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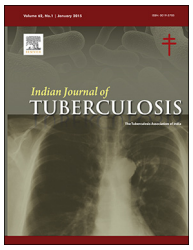

Editorial

GeneXpert: A momentous innovation that needs a touch of prudence

"It has been more than 134 years since Robert Koch[1] announced the discovery of *Mycobacterium tuberculosis* (*M. tuberculosis*) to a stunned world. In spite of effective chemotherapy being available for more than 60 years, the scourge of tuberculosis (TB) continues to claim more lives due to a curable infectious disease than is acceptable in the 21st century. From 2016, the World Health Organization (WHO) aims to end the global epidemic of TB by implementing the "End TB strategy". This target will serve as a blue print for countries to reduce the number of deaths due to TB by 90% by 2035, reduce new cases by 80% and ensure that no one is burdened with the enormous cost of treatment of TB.[2]

To achieve this goal, we also need newer diagnostics that are simple, accurate and establish the diagnosis rapidly.[2] Sputum smear microscopy continues to remain the pivot for the diagnosis of pulmonary TB even now. Culture, though the gold standard, is time consuming and labour intensive. The need for simple, accurate and rapid diagnostic methods has led to the development of molecular assays and in particular the Xpert MTB/RIF (GeneXpert MTB/RIF, Cepheid Sunnyvale, California). The advent of this particular test has resulted in a paradigm shift in the way TB is being diagnosed.

The Xpert MTB/RIF offers an efficient and robust near patient technology that can rapidly detect *M. tuberculosis* and rifampicin (RIF) resistance with high accuracy. Such a molecular test that is simple enough to be used in regions other than conventional laboratory settings has been used for the first time. The Xpert instrument can be placed anywhere from high throughput reference laboratories to peripheral clinics but would be dependent on the availability of infrastructure and trained personnel.[3] Xpert MTB/RIF detects *M. tuberculosis* as well as RIF resistance, a surrogate marker of multidrug resistant tuberculosis (MDR-TB) using three specific primers and five unique molecular probes ensuring a high degree of specificity in a fully automated DNA testing platform.[3,4] The probes are complementary to a 81 bp core region of the *rpoB* gene. Mutations in this region are responsible for 95% of the cases of RIF resistance. The assay provides results directly from sputum in less than 2 h. It is more sensitive than smear microscopy with a limit of detection (LOD) of 131 CFU/ml of sputum.[4] In addition, the assay's sample reagent, used to liquefy sputum, has tuberculocidal properties that eliminate biosafety concerns to a large extent.[5]

The WHO has stated that when used as an initial diagnostic test in place of smear microscopy, the Xpert MTB/RIF achieved an overall pooled sensitivity of 88% and pooled specificity of 99%.[6] The WHO currently recommends the use of Xpert MTB/RIF in preference to conventional bacteriological techniques, as an initial diagnostic test for diagnosing pulmonary TB in adults and children suspected of having MDR-TB or human immunodeficiency virus (HIV) associated TB. In view of the resource implications, conditional recommendation allows the use of Xpert MTB/RIF as an initial diagnostic test in all adults and children with a clinical suspicion of TB. However, the quality of evidence available for the paediatric age group is rather low.

Similarly, the quality of evidence for extrapulmonary TB remains low. The pauci-bacillary state of extrapulmonary TB is often responsible for diagnostic predicament. The sensitivity of the test for detection of *M. tuberculosis* in pleural fluid when compared with culture is reported to be as low as 43.7%.[3] The recent Index-TB guidelines, an initiative of the Central TB Division, Ministry of Health and Family Welfare, Government of India, recommended that Xpert MTB/RIF should not be used to diagnose pleural TB.[7] Data for other types of extrapulmonary specimens including ascitic fluid, pericardial fluid, urine, blood and stool are limited.[3] For cerebrospinal fluid, it is recommended that the Xpert MTB/RIF may be used as the initial diagnostic test for tuberculous meningitis.[6,8] However, using Xpert MTB/RIF as the sole diagnostic assay in tuberculous meningitis may lead to missed cases.[8] Although, there is very low quality of evidence, Xpert MTB/RIF may be used as a replacement test for microscopy, culture and histopathology for testing specific extra pulmonary specimens such as lymph nodes.[6,7] The WHO has recommended that the algorithms guiding the Xpert MTB/RIF test should be based on the available resources and anticipated cost benefit in each country.

The Xpert MTB/RIF was first used in India as part of a demonstration project in reference laboratories in 2010. At the

end of the 2 year project, the Xpert was first launched in Sion Hospital, Mumbai on March 24, 2012[9] and this was followed by a rapid increase in the use of Xpert TB/RIF in the country. The Revised National Tuberculosis Control Programme (RNTCP) endorses the Xpert MTB/RIF for diagnosis of MDR-TB among MDR-TB suspects[10] and the induction of the assay in the RNTCP, is expected to impact and diminish the progress of MDR-TB due to its inherent ability to provide identification and DST results for RIF simultaneously.[11] The RNTCP recommends that the Xpert should be the preferred first diagnostic test in children and in patients with HIV.[10] Furthermore, the guidelines state that for presumptive extrapulmonary TB, appropriate specimens from the site of involvement should be used for Xpert and other microbiological tests.[10]

The overall negative predictive value of the Xpert MTB/RIF is an estimated 98% and a negative test, in an appropriate setting, would accurately exclude TB in most situations.[3] Poor quality of the sample collected would result in a negative Xpert MTB/RIF but a patient with strong clinical suspicion of having TB would require repeat investigations. The specificity of Xpert MTB/RIF being high (99%), DNA from dead bacilli may be detected giving a false positive result. However, the culture under these circumstances would be negative.[3] The Xpert MTB/RIF also has a high sensitivity in detecting RIF resistance. A negative result for RIF resistance would accurately exclude the presence of resistance to RIF. However, rarely, a patient may be suspected of having MDR-TB but have a negative result from Xpert MTB/RIF. In such a case, a follow up test may be done using a phenotypic drug susceptibility assay.[3] The positive predictive value (PPV) of Xpert MTB/RIF for detecting RIF resistance is more than 90% in regions where the prevalence of RIF resistance is higher than 15%. In populations with low prevalence of RIF resistance, the PPV would also be low, hence, the emphasis on targeted testing to improve the PPV.[3]

Several investigators have evaluated and validated the use of Xpert MTB/RIF in clinical specimens.[4,12] A comparison between Xpert MTB.RIF and the Line probe assay, has shown similar sensitivity and specificity for both assays.[13,14] In India, investigators have evaluated the Xpert MTB/RIF for pulmonary and extrapulmonary specimens.[15,16] Singh et al.[15] demonstrated a sensitivity and specificity of 100% in pulmonary samples, on comparing the Xpert MTB/RIF with composite reference standards. The sensitivity and specificity for extrapulmonary samples were 87.5% and 81.82% respectively. Rufai et al.[16] investigated samples of pleural fluid and obtained a sensitivity of 54.8% and specificity of 100% in comparison to MGIT-960 culture system. More recently, Agrawal et al.[17] performed a comparative study of Xpert MTB/RIF with Ziehl Neelsen staining. They demonstrated a higher sensitivity of Xpert MTB/RIF as compared to Ziehl Neelsen staining in respiratory samples. A recent study conducted in 18 subdistrict level TB programme units in India concluded that the introduction of Xpert MTB/RIF as initial diagnostic test for TB in public health facilities significantly increased case notification of bacteriologically confirmed TB by 39% and RIF resistant TB by fivefold.[18]

The major limitation of Xpert MTB/RIF is that it can only detect resistance to RIF. The *rpoB* allele which is responsible for RIF resistance should be present in at least 65% of DNA in the sample.[19] The earlier version of cartridges also had limitations in reporting Leu533Pro mutations that could be missed unless 100% of the DNA population in the sample had the mutation.[19,20] However, the G4 version of the assay included several modifications intended to improve detection of mutations at codon 533.[21] In regions where TB is endemic, mixed infections with both drug-sensitive and drug-resistant strains of *M. tuberculosis* have been reported.[22] This assay has an increased false-negative rate for detecting RIF resistance with mixed *M. tuberculosis* complex infections and may need further confirmation. The assay does not work well for monitoring of patients developing RIF resistance during the course of treatment.[19] The availability of Xpert MTB/RIF does not eliminate the need for microscopy and culture, since both would be required to follow up patients already on treatment. In addition, culture is required to perform drug sensitivity testing against antituberculous drugs other than RIF. A next-generation cartridge called Xpert Ultra® has also been developed. It is intended to replace the Xpert MTB/RIF cartridge and could potentially replace conventional culture as the primary diagnostic tool for TB.[2]

However, to achieve the ''End TB goal'', involvement of the private sector is a must and the complex healthcare system of India must also be taken into account. The biggest challenge in a scale up of Xpert MTB/RIF in the public sector for us would be providing adequate funds, training of personnel in the peripheral microscopy centres and establishing infrastructure like uninterrupted power supply. The Xpert MTB/RIF, in our country is available at a special negotiated price in the public sector but the private sector does not benefit from this reduced cost.[23] Till the cost of Xpert MTB/RIF is brought down uniformly or a cheaper version is available, use of rapid technology in the peripheral regions for diagnosis in TB will not be wholly met.

To conclude, the advent of the Xpert MTB/RIF has led to a revolution in the diagnostics landscape of TB. Such a consumer friendly yet rapid, sensitive and specific assay has never been used before. However, given the limitations of the assay including false positive results in previously treated patients due to presence of dead bacilli and false negative results in mixed infections, the Xpert TB/RIF should be used with caution. In order to determine accurately the diagnostic efficacy of the Xpert MTB/RIF, especially in paucibacillary cases, more elaborate studies would be needed from countries with low-income and high tuberculous burden. We are not yet in a position to discard the conventional smear microscopy and blindly following the Xpert MTB/RIF without taking into account the recommendations laid down by WHO could prove to be counter-productive.

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Viewpoint

LAMP – An innovative POC tool for diagnosing pulmonary TB in remote areas

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Tuberculosis (TB) is still a leading global killer. Worldwide 2–3 billion people are infected with TB, an infectious disease which is caused by bacteria belonging to the *Mycobacterium Tuberculosis Complex* (*MTBC*). 10.46 million people became ill of TB and 1.8 million people died in 2015, including 0.4 million TB death among HIV positive people.[1] From 2016, the goal is to end the global TB epidemic by implementing the End TB Strategy. Over 95% of all TB deaths occur in low and middle income countries. In 2015, millions of new cases of TB were reported to WHO, fewer than two-thirds of 10.4 million people estimated to have fallen sick with the disease. This means that worldwide, nearly 3–4 millions of new cases went undiagnosed or were not reported.[1] The most common method for diagnosing TB is sputum smear microscopy by carbol fuchsin or fluorescence staining. Although the specificity is quite good with 98–100%, it means that nearly every second positive case is not detected with smear microscopy. The poor sensitivity and reliability of smear microscopy need other test methods to confirm negative or positive results. This confirmation testing is part of all published WHO testing algorithms. The reference method for diagnosing TB is bacterial culture that was recommended by WHO 2006. Beside the good test performance, the time from sample to result is high (days-weeks) and in most areas with resource limited settings not feasible. Although nucleic acid amplification (NAA) methods are available for first and second-line *MTBC* testing, most of these tests require well-equipped laboratories and personnel and are difficult to realize in remote areas due to the missing logistics and reliable availability of electricity. Thus, the challenge of diagnosing TB in resource-limited settings lies in the fact that sensitive, fast and cost effective assays are missing which are easy to perform and that have lower demands in logistics.

TB-LAMP is a molecular test that bases on the loop-mediated isothermal amplification (LAMP) for the detection of *MTBC* and was recommended by WHO in August 2016. The technology was developed and published by Notomi et al.[2] Compared to MTBC detection by real-time PCR, LAMP takes less than an hour (depending on sample number) and is performed only with two incubators. These two incubators have preinstalled and fixed incubation times and temperatures and are part of the HumaLoop T. The instrument also contains the fluorescence detection unit for result reading and has a capacity of 16 tests. The TB-LAMP workflow consists of three steps:

1. Easy sample transfer with Pipette-60

   Only 60 μl sputum sample is necessary to perform TB-LAMP. Due to its viscosity, it's often difficult to transfer sputum samples. The Pipette-60 comes with special filter tips that ease the sample transfer into the heating tube. The LAMP method is that flexible that the assay can be performed with minimum 45 μl and maximum 75 μl sample volume.
2. Ultrafast DNA purification with the Loopamp™ PURE DNA Extraction Kit

   After transfer of the sputum sample into the blue colored lysis buffer that is in the heating tube, the sample is lysed for 5 min at 90 °C. Then, the lysis tube is connected with an absorbent tube. This tube contains powder that removes all of the inhibitors that could disturb the amplification reaction. During this process, the lysed sample runs into the powder and the entire PURE tube is mixed through roughly until a milky solution is obtained. Finally, the PURE tube is connected with an injection cap and the DNA solution can be extracted manually by squeezing directly into the LAMP reaction tube (Figs. 1 and 2).

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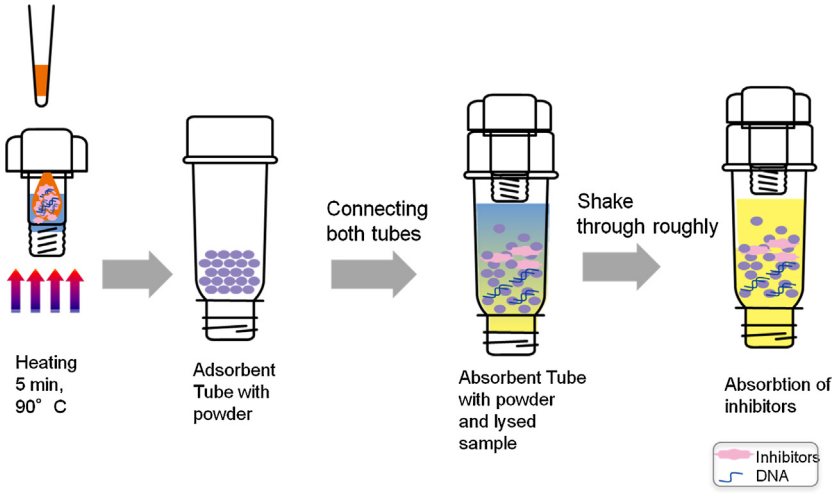

Fig. 1 – Sample transfer and Loopamp™ PURE treatment.
***Source*: Human, Gesellschaft für Biochemica and Dagnostica GmbH, Germany.**

3. LAMP reaction with the Loopamp™ MTBC Detection Kit

The LAMP reaction tube contains dried, colored reagents in the tube cap. These features have different advantages: Firstly, the kit can be shipped at 1–30 °C and does not need any cold chain (easy logistics). Secondly, the colored reagents are reconstituted with the extracted DNA solution by 2 min incubation and several mixing steps so that the color goes into solution once all of the reagents are solved and the cap becomes blank.

Another advantage of LAMP is the volume of the DNA solution which is required to perform LAMP. Each tube contains two lines that indicate the minimum (25 μl) and maximum (35 μl) amount of the required DNA solution. Under optimum conditions, the liquid that is squeezed out is in between these two lines but the assay works within the given volume range (Fig. 3).

After the incubation and mixing steps, the reaction mix is completed and ready for the amplification reaction. It is performed for 40 min at 67 °C in the second chamber of the HumaLoop T. During this step, the quenching of the fluorescence dye Calcein is canceled and fluorescence light is generated. Another byproduct of the amplification reaction is the generation of manganese pyrophosphate that makes the reaction solution turbid. After the amplification, HumaLoop T heats up to 80 °C and during a 5 min incubation step the amplifying enzymes are inactivated and the reaction is terminated by a beep.

1. Result interpretation

To see the results, the reaction tubes are transferred into the fluorescence detection unit that contains a UV light source (LED). With the positive and negative control that should be performed with each run, the results can be easily interpreted: a MTBC positive result shows a green fluorescence, a MTBC negative result has no fluorescence (Fig. 4).

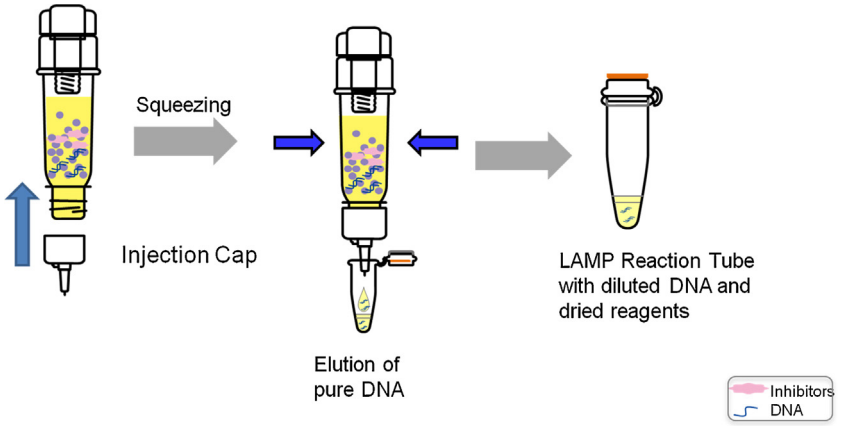

Fig. 2 – Extraction of DNA with the Loopamp™ PURE DNA Extraction Kit.
***Source*: Human, Gesellschaft für Biochemica and Dagnostica GmbH, Germany.**

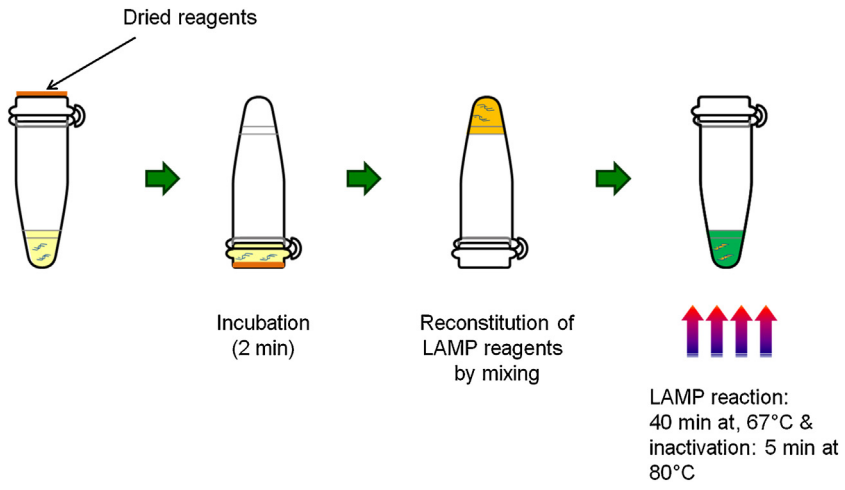

Fig. 3 – LAMP reaction.
***Source*: Human, Gesellschaft für Biochemica and Dagnostica GmbH, Germany.**

In conclusion, the TB-LAMP is very easy to perform and adaptable to all levels of laboratories, but especially to remote areas.

1.1. *Test performance*

So far, the performance of TB-LAMP in resource limited settings where smear microscopy is performed as preferred first-line test, has been evaluated with more than 4000 sputum samples. A summary of recent published studies is shown in Table 1.[3–6]

Summarizing this data, the obtained sensitivities of TB-LAMP ranged from 92.1% up to 100% in smear+/culture+ sputum samples and from 52.1 up to 90.3% in smear−/culture+ samples from suspected TB patients. The specificities ranged from 96.6% up to 100% in new patients. Significantly lower specificities were detected in sputum samples from antibiotic treated patients. In all studies, TB-LAMP had a significant better test performance than smear microscopy. Kaku et al. outlined that the use of the LAMP-TB test could increase the detection of TB by 18% in comparison to LED microscopy. These data correlate with their preliminary study result in Haiti.[7] The test performance of PURE-LAMP TB in smear−/culture+ sputum samples is comparable to the GeneXpert system, a WHO endorsed test. Data from 21 studies, where the Xpert® TB/RIF system was used as add-on test showed 67% sensitivity and 98% specificity in smear−/culture+ samples (based on 15 different reports).[8]

1.2. *Challenges of TB-LAMP*

Like every diagnostic test, TB-LAMP has its challenges.

1. With TB-LAMP RIF resistance cannot be detected. RIF is one of the most effective first-line anti-TB antibiotics and together with isoniazid constitutes the basis of the multidrug treatment regimen for TB. Rifampicin is active against growing and non-growing (slow metabolizing) bacilli. TB-LAMP was initially developed to replace smear microscopy. Especially in countries with high MDR prevalence in new and retreated patients is RIF testing is required as first line testing. Although RIF testing with the GenXpert system was reported to provide some false positive results, first line resistance testing should be performed if available.[9–10] Beside RIF resistance, other resistances become more and more apparent. Nearly 10% of all MDRs are XDRs and the mono resistance of RIF is rare. Therefore, DST testing is necessary for an efficient therapy and a positive RIF result does not replace DST from culture.
2. The WHO estimates in 2015 that 1 million children (<15 years), currently suffer from TB and that more than 136,000 die each year.[1] TB-LAMP was not fully evaluated in samples

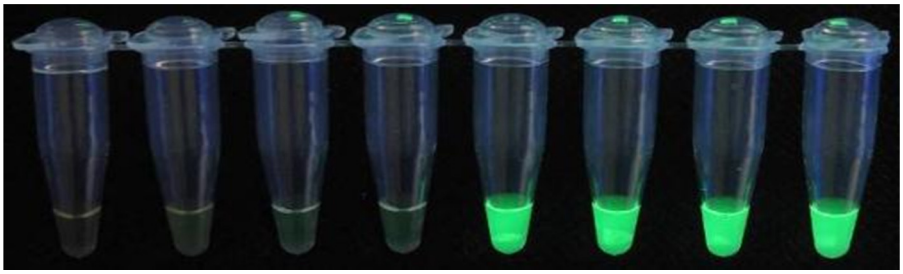

Fig. 4 – Results of 4 negative samples (left) and 4 positive samples (right). The LAMP reaction was performed with HumaLoop T.
***Source*: Eiken Chemicals Ltd., Tokyo.**

Table 1 – Data of recent published studies outlining the excellent test performance of TB-LAMP.

PURE-LAMP-TB	N	Sensitivity Smear+	Sensitivity Smear−	Specificity (Culture−)	Treatment status
Ou et al. (2014)[3]	1329	92.1% (152/165)	53.8% (113/210)	98.3% (938/954)	Before (spot sputum)
		88.8% (333/375)		96.8% (924/954)	Before (spot/morning/night sputum)
Kaku et al. (2016)[4]	472	99.1% (113/114)	52.1% (21/41)	98.4% (312/317)	Before (sample analysis)
	209	100% (47/47)	56.5% (13/23)	97.8% (136/139)	Before (patient analysis)
Gray et al. (2016)[5]	1745	97.2% (243/250)	62% (88/142)	96.6% (1307/1353)	Before
Bojang et al. (2015)[6]	261	100%	90.3%	100% (Smear+); 99% (Smear−)	Before
	156	100%	71.3%	63% (Smear+); 93% (Smear−)	Follow up

for children, yet. Due to the principle of LAMP and the robustness of the assay it might be an advantage that only 60 μl sample are required for TB-LAMP testing instead of 1 ml that is needed for Xpert TB/RIF testing.

3. In the policy guide, WHO excluded all data were obtained from extra pulmonary samples and the validation of TB-LAMP testing with extra pulmonary samples is still under investigation.[11] Preliminary internal data obtained from are very promising. Together with the small sample volume, TB-LAMP might be a helpful tool for diagnosing TB in extra pulmonary samples, especially from children.
4. Similar to the data in children, TB-LAMP was not fully evaluated in HIV patients, yet. HIV infection and TB are co-occurring epidemics. Unfortunately, diagnosis of TB is challenging in this population because concurrent HIV infection is associated with sputum smear-negative disease and a higher proportion of extra pulmonary TB.[12] First data show a decreased test performance of TB-LAMP in HIV patients.[11] But further studies need to be done in low and high endemic settings compared to the Xpert® TB/RIF assay.
5. An increase of false positive results with TB-LAMP were observed using negative controls in labs with a higher temperature ≥ 37 °C and with 25 μl eluent were used. Examination of the retained sample reaction tubes suggested that the false positivity may have been related to adding too little of the DNA eluent from the PURE method to the reaction tube (20–25 μl). Running TB-LAMP with only the negative control, addition of 20–25 μl eluent into the reaction tubes produced a 1% false positive rate (FPR) (4/400 tests). Reaction tubes were then exposed to varying temperatures (25, 37, 40 and 45 °C) and high humidity (85–95%) for 15 min prior to adding 25 μl or 30 μl eluent to the reaction tubes. False positives only occurred at 40 and 45 °C and with 25 μl added (5/96, 5.2% FPR). No other false positives were detected.[5,13] Nevertheless, the GeneXpert System cannot be used due to limited working temperature range up to 30 °C of the GeneXpert instrument.[14] The HumaLoop T instrument provides a better temperature "resistance" up to 40 °C.
6. The situation in resource limited settings are characterized by the lack of sustained electricity and that there is no access to case registration. Like the GeneXpert system, TB-LAMP needs constant electricity. Longer outages of one to several days were also reported in resource limited settings. Although a UPS is recommended for each instrument, the instrument cannot be operated with batteries. On-going investigations will outline new opportunities to operate HumaLoop T with solar panels or the usage of other alternative energy source independent form the local electricity. Result report ability with the TB-LAMP system can be only provided with the HumaTurb system which provides LIS and USB connectivity. In primary settings where HumaLoop T is used, the results cannot be send to e.g. central labs and need to be documented in a written form.
7. Like other molecular tests, TB-LAMP cannot be used for the monitoring of therapy as it detects dead and live bacteria. Therefore bacterial culture is needed to evaluate the success of TB therapy.

2. Conclusion

LAMP combines the test performance known form other molecular methods, robustness, easy logistics by transportation at 1–30 °C and minimum training efforts. Furthermore there is no need for additional equipment that reduces further investments. These features render LAMP to a simple and useful tool for diagnosing *MTBC* in peripheral health centers where smear microscopy is performed. The replacement of smear microscopy by TB-LAMP in remote areas offers the opportunity to detect TB much earlier and more reliable than smear microscopy. TB-LAMP reduced the cases of undiagnosed TB patients, leads to an earlier targeted therapy and helps to achieve the WHO goals to eliminate TB. Further implementation and cost effectiveness studies will clarify the importance of TB-LAMP as new POC tool for diagnosing pulmonary TB in remote areas.

Conflicts of interest

The author has none to declare.

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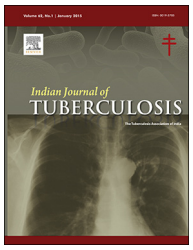

Original Article

Study of the structure and functioning of referral mechanism of patients receiving treatment and records linkage under Revised National Tuberculosis Control Programme (RNTCP) of Government of India

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ABSTRACT

Background: The reliable and successful performance of the Revised National Tuberculosis Control Programme (RNTCP) ''referral mechanism'' is profoundly important in the medical college scenario, and it is an important requirement of the programme to have feedback status report of the referred patients.

Methods: An observational study on tuberculosis (TB) patients referred from Directly Observed Treatment (DOT) Centre, Sri Venkateswara Institute of Medical Sciences (SVIMS) was conducted during the years 2010 to 2012 ($n = 622$). Subjects referred to other TUs within the District but failed to report there within 45 days constituted ''cases'' and subjects, who obtained treatment from the TUs they were referred to ''controls''. The initial information or confirmation of registration for treatment status feedback were obtained from patient/Senior Treatment Supervisor (STS)/District Tuberculosis Centre (DTC) levels respectively both before using intervention (Phase I, year 2010) and after using intervention (Phase II, years 2011 and 2012) by sending day-to-day text messaging of referral details of patients to the STS and District Tuberculosis Officer (DTO).

Results: During Phase I, the distribution of subjects ($n = 242$) in the ages ≤25, 26–50, and ≥51 years was similar in both the cases and control subjects ($p = 0.054$). Further, there was no statistically significant difference in the median age of the cases and controls [34.5 (interquartile range, IQR 31–51) vs 39 (30–54); $p = 0.319$]. There was no statistically significant difference in other parameters, such as gender distribution ($p = 0.9748$); availability of phone numbers ($p = 0.9614$); type of disease ($p = 0.8395$); and type of case ($p = 0.0793$). In Phase II, the effect of intervention on feedback related parameters showed statistically significant improvement in all the parameters such as initial feedback levels obtained within 15 days ($p = 0.0077$); within 45 days ($p < 0.0001$); above 45 days ($p < 0.0001$); registration status confirmation within 45 days ($p = 0.0343$); mismatch of feedback received by observer ($p < 0.0001$); and telephone number of patients recorded ($p < 0.0001$).

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Conclusion: Our findings suggest that text messaging reminders may be an important tool to achieve optimal feedback response in resource-limited settings.

1. Introduction

The World Health Organization (WHO) 2015 Global TB Report states that world over, an 9.6 estimated million people were infected with TB and 1.1 million died from the disease.[1] The Government of India (GOI) launched RNTCP in 1997 and by March 2006 whole of India was covered by the programme, adopting the internationally recommended DOTS strategy[2] free of cost at their place of domicile.

There is an inbuilt referral feedback mechanism in the RNTCP for tracking patients who are referred for treatment to their place of domicile. This mechanism enables to provide feedback to the referred unit so as to ensure that the referred patients are initiated on treatment and registered under RNTCP.[3,4] RNTCP over the last decade has undertaken the unique exercise of involving medical colleges in TB control. This is the first-time a public health programme has successfully involved medical colleges in this manner.[5] Medical college teaching hospitals are tertiary care referral centres, and patients from far-flung areas are referred to these hospitals for diagnosis confirmation and expert management. Therefore, reliable and successful performance of the RNTCP ''referral mechanism'' is profoundly important in the medical college scenario than at any other peripheral health institution (PHI) under the RNTCP.

In spite of a strong structure, patients, who are diagnosed in this health system, are getting lost as the receiving units failed to provide feedback regularly. In a medical college scenario, reliable data regarding the feedback status regarding a patient referred for treatment from a medical college teaching hospital are lacking in published literature. The present study, therefore, was designed to study the performance of the existing referral mechanism under the RNTCP, identify the problems related to the same and also explore the feasibility of devising and instituting remedies for the same. The study on the present scenario would suggest linkages of referral information so as to strengthen the existing referral mechanism in vogue under RNTCP.

2. Materials and methods

All patients diagnosed to have various forms of TB at the designated microscopy centre (DMC) and Directly Observed Treatment (DOT) Centre at SVIMS, Tirupati who were referred for treatment between January 2010 and December 2012 were included in this observational study. Patients satisfying the following criteria were included in the study: patients referred from SVIMS DOT Centre to other treatment units (TUs) within the Chittoor District, patients referred to SVIMS DOT Centre from within the Chittoor District, patients from outside the Chittoor District, who fall within the catchment area of DOT Centre, SVIMS referred to SVIMS DOT Centre. Patients referred from SVIMS DOT Centre to TUs outside the Chittoor District and other States, patients opting to avail treatment at private practitioners and Non-Governmental Organizations (NGOs) in the study were excluded. The study was cleared by the Doctoral and Institutional Ethics Committees.

The study was carried out in two phases. During Phase I of the study (year 2010), all the subjects who were diagnosed at DMC, SVIMS and started on DOTS and referred to other TUs within the District but failed to report to their TUs within 45 days from the date of referral were classified as cases and all subjects, who obtained treatment from the same TUs they were referred to classified as controls.

During the entire study period, as per RNTCP norms ''referral for treatment forms'' in triplicate were used to obtain feedback using the postal system to the respective DTO (Form A), health facility where the patient is referred to (Form B) and the patient (Form C). In Phase II, (year 2011 and 2012) an intervention was designed to improve the performance of the referral feedback mechanism, and the study was conducted to assess the efficacy of this intervention. Since postal referral would take longer time, a text message using the short message service (SMS) was sent with the intention to communicate rapid and early.

2.1. *Intervention*

As an intervention, day-to-day SMS on the details of the referred patients had been sent through mobile phones to the STSs concerned and DTO on the day of referral itself, in order to reduce the communication gap and trace the patient earlier than that of postal system. In addition, monthly list of referred patients with details were sent to 'DTC-email' along with a hard copy sent through TB Health Visitor (TBHV), SVIMS every month prior to the monthly co-ordination meets as followed during the Phase I of study.

2.2. *Data collection*

The quality assurance was ensured by adopting following measures: to establish contact with the referred patient within 10 days from the date of referral, to ensure patients registered at their TU within 14 days from the date of referral, to provide feedback to the DTO on non-traceable patients, and provide correct surveillance report during the monthly meetings with STS's.

2.3. *Monitoring progress*

In addition, the method of monitoring adopted in Phase I was also followed in Phase II. On a daily basis, the DOT Centre at SVIMS, Tirupati was checked for verifying whether any postal feedback was received from the respective receiving units for

the patients referred for treatment. The data of referral register at DOT Centre, SVIMS, Tirupati were studied for the period 2010 to 2012 and 1330 TB patients were diagnosed out of which 1121 cases were referred. As per inclusion criteria, 622 cases referred within Chittoor district were included in two phases of the study. The number of cases tracked for feedback were: first phase (year 2010) 242 out of 435 before using intervention; first year of Phase II (year 2011) 184 out of 339 cases; and second year of Phase II (year 2012) 196 out of 347 cases after using intervention.

2.4. *Statistical analysis*

The day-to-day referral data were updated in Microsoft Excel 2007 (Microsoft Corp, Redmond, WA) format with date of receiving feedback forms. The information received from the patient, STS and DTC-email had been classified under PI, SI and EI fields and confirmation of the cases getting registered, had been classified under PT, ST and ET fields respectively. Descriptive statistics for the categorical variables were performed by computing the frequencies (percentages) in each category. Variables following normal distribution were summarised by mean and standard deviation; the remaining variables were summarised as median [interquartile range (IQR)]. The association between two categorical variables was evaluated by Chi-square test (χ^2). Mann–Whitney *U* test was used to compare continuous variables which do not follow normal distribution between the groups. Statistical analysis of 622 patients and their feedback obtained from the patient, STS and DTC-email was been compared between the groups of patients referred before and after using intervention. Statistical analysis was carried out using statistical software IBM SPSS, Version 20, (IBM SPSS Statistics, Somers NY, USA).

3. Results

During the period January 2010 to December 2012, 1330 TB patients were diagnosed of which 1121 were referred for treatment from SVIMS DOT Centre. Among the referred group, a total of 622 patients after satisfying inclusion criteria were recruited in the study, mobile phone numbers could be obtained from 542 (87.1%) patients/their next responsible attendants. One patient (0.2%) possessed only a landline phone; and the remaining 79 (12.7%) patients/their attendants did not possess a mobile/landline phone.

3.1. *Cases vs controls feedback pattern before intervention during Phase I*

Comparison of characteristics of cases and control subjects ($n = 242$) seen during the year 2010 (Phase I of the study) is shown in Table 1. The distribution of subjects in the ages ≤25, 26–50, and ≥51 years was similar in both the groups ($\chi^2 = 5.833$, $p = 0.054$) (Table 1). Further, there was no statistically significant difference in the median age of the cases and controls [34.5 (IQR 31–51) vs 39 (IQR 30–54); $p = 0.319$]. There was no statistically significant difference in other parameters, such as sex distribution ($\chi^2 = 0.001$, $p = 0.9748$); availability of phone numbers ($\chi^2 = 0.006$, $p = 0.9614$); type of disease ($\chi^2 = 0.041$, $p = 0.8395$); and type of case ($\chi^2 = 3.08$, $p = 0.0793$) shown in Table 1.

Patients in age group of 26–50 years were maximum (47%), and distribution in age group of below 25 years (23%) and above 50 years (50%) were similar. Of which majority of the subjects, that is, 95 (29%) were in the control group between the age of 26–50 years. Most of the patients, that is, 138 (57%) were men and they outnumbered women in both the groups (57%:18% vs 10%:5%). A higher percentage of men, that is, 138 (57%) were in control group.

Majority, that is, 196 (81%) landline phone numbers/mobile phones of the patients/their next responsible attendants were collected, and 166 (69%) were in control group. Most of the patients ($n = 160$) had extra-pulmonary TB than pulmonary TB ($n = 82$); of which a predominant percentage of subjects (56% vs 29%) were in control group. The type of cases categorised were: 'new': 206 (85%); and 'previously treated': 36 (15%). A majority

Table 1 – Comparison of characteristics of cases and control subjects seen during the year 2010 (Phase I of the study).

Characteristics		Status		Total ($n = 242$)	Significance difference
		Controls ($n = 205$)	Cases ($n = 37$)		
		No. (%)	No. (%)	No. (%)	
Age group (years)	≤25	52 (22)	3 (1)	55 (23)	$p = 0.054$
	26–50	95 (39)	19 (8)	114 (47)	
	≥51	58 (24)	15 (6)	73 (30)	
Sex	Male	138 (57)	25 (10)	163 (67)	$p = 0.9748$
	Female	67 (28)	12 (5)	79 (33)	
Phone numbers collected	Yes	166 (69)	30 (12)	196 (81)	$p = 0.9614$
	No	39 (16)	7 (3)	46 (19)	
Type of disease	PTB	70 (29)	12 (5)	82 (34)	$p = 0.8395$
	EPTB	135 (56)	25 (10)	160 (66)	
Type of case	New	178 (73)	28 (12)	206 (85)	$p = 0.0793$
	Previously treated	27 (11)	9 (4)	36 (15)	

PTB = pulmonary tuberculosis; EPTB = extra-pulmonary tuberculosis; No. = number.

Table 2 – Effect of intervention on feedback related parameters.

Parameters		Intervention		Result
		Before (year 2010)	After (year 2011–2012)	
Initial feedback levels obtained within 15 days (PI/SI/EI data)	Patient	113/242 (46.7%)	236/380 (62.1%)	$p = 0.0077$
	STS	34/242 (14.1%)	137/380 (36%)	
	DTC-email	11/242 (4.6%)	19/380 (5%)	
Initial feedback levels obtained within 45 days (PI/SI/EI data)	Patient	153/242 (63%)	144/380 (37.9%)	$p < 0.0001$
	STS	159/242 (65.7%)	327/380 (86.1%)	
	DTC-email	67/242 (27.7%)	109/380 (28.7%)	
Initial feedback levels obtained above 45 days (PI/SI/EI data)	Patient	4/242 (1.7%)	8/380 (2.1%)	$p < 0.0001$
	STS	83/242 (34.3%)	53/380 (14%)	
	DTC-email	175/242 (72.3%)	271/380 (71.3%)	
Registration status confirmation (ST/ET data) – within 45 days	STS	139/203 (68.5%)	277/343 (80.8%)	$p = 0.0343$
	DTC-email	67/203 (33%)	89/343 (25.9%)	
Mismatch of feedback received by observer	Yes	36/242 (14.9%)	109/380 (28.7%)	$p < 0.0001$
	No	206/242(85.1%)	271/380 (71.3%)	
Telephone number of patients recorded	Yes	196/242 (81%)	347/380 (91.3%)	$p < 0.0001$
	No	46/242 (19%)	33/380 (8.7%)	

PI = patient information of reporting to the system/status of the patient; SI = Senior TB Treatment Supervisor confirming the patient reporting information in to the system; EI = DTC-email confirming the information of patient status provided by the Senior TB Treatment Supervisor; PT = patient confirming their TB number provided; ST = Senior TB Treatment Supervisor confirming the TB number provided; ET = DTC-email confirming the TB number of the patient; TBHV = TB Health Visitor; STS = Senior TB Treatment Supervisor; DTC = District Tuberculosis Centre; TU = tuberculosis unit; *n* = number; TB = tuberculosis.

178 (73%) of 'new' cases were in control group compared to 38 (12%) in cases.

3.2. *Effect on parameters measured before and after intervention*

The effect of intervention on feedback related parameters is shown in Table 2. There was statistically significant change in all the parameters such as initial feedback levels obtained within 15 days ($\chi^2 = 9.728$, $p = 0.0077$); within 45 days ($\chi^2 = 27.44$, $p < 0.0001$); above 45 days ($\chi^2 = 20.652$, $p < 0.0001$); registration status confirmation within 45 days ($\chi^2 = 4.476$, $p = 0.0343$); mismatch of feedback received by observer ($\chi^2 = 15.77$, $p < 0.0001$); and telephone number of patients recorded ($\chi^2 = 14.21$, $p < 0.0001$) (Table 2).

Following the intervention, there was a significant increase in the initial feedback levels obtained from date of referral at patient/STS/DTC-email after intervention, within 15 days; within 45 days; and above 45 days. At the STS level, the effect of intervention had shown improvement in providing initial feedback within 45 days and had also showed a decreasing trend in above 45 days.

The referred patients registration status had also shown to have been benefited by using intervention except at patient level. The registration status confirmation within 45 days has been influenced significantly using intervention. At both STS and DTC-email level, the confirmation of patients registered in RNTCP system had increased.

There were 206 'no mismatch' before intervention. It had increased to 271 after intervention. The collection of landline telephone/mobile phone number of patients/their next responsible attendants had improved after intervention. There were 196 cases of phone numbers recorded before intervention, and it had gone up to 347 after intervention.

4. Discussion

The RNTCP aims at providing DOTS treatment to patients with TB from a PHI/DOT provider at the place of domicile of the patient. A well laid-out ''referral and feedback system'' is considered to be a strong point of RNTCP management system.

The importance of the referral-feedback mechanism is particularly highlighted in the context of the medical colleges. This is because, medical colleges being tertiary care referral centres, attract patients from far-flung areas for diagnostic work-up and not surprisingly, a majority of patients diagnosed to have TB at a medical college under RNTCP are referred to their place of domicile for treatment. Sparse published data are available regarding the referral-feedback obtained under real-life situations under field conditions at medical colleges in India. In a National Task Force (NTF) meeting, it was mentioned that the feedback to the referring medical college regarding treatment initiation was received from 73% sputum positive cases; 68% of sputum negative pulmonary TB cases and 62% extra-pulmonary TB (EPTB) cases.[6] However, wide regional variations are known with regard to these parameters.

The Phase I of the study also brought into light several other operational issues such as no effective surveillance established on postal feedbacks at DTC level and no feedback was received from referred centre/DTC; dispatch of referral forms to referred centres was delayed that interfered with referral-feedback mechanism. The feedback mechanism appeared to be addressed only during monthly co-ordination meetings. The characteristics of cases and control subjects were similar (Table 1). The median age of cases and controls [34.5 (IQR 31–51) vs 39 (IQR 30–54); $p = 0.319$] (Table 1); other parameters such as gender distribution ($p = 0.9748$); availability of phone

numbers ($p = 0.9614$); type of disease ($p = 0.8395$); and type of case ($p = 0.0793$) noted (Table 1), showed no statistically significant difference between the cases and controls. Observations made during this study suggest that in real-time, the postal feedback system is functioning sub-optimally. Recognising the non-functioning postal referral feedback system an ''SMS based intervention'' was conceived and employed in the present study.

After institution of intervention during the year 2011, the time delay from STS for the referred patient information had reduced considerably, and the patients tracked into the RNTCP system within first 15 days from date of referral had increased from 14% (before intervention period) to 36% (Table 2) ($p = 0.0077$). There was a statistically significant increase in registration status confirmation within 45 days ($p = 0.0343$) (Table 2).

The observations from an earlier study[7] suggested that the delay in receipt of feedback from referring units correlated with the changes/transfer of the RNTCP contractual staff. Better performing TUs too had effectively utilised the intervention and had raised their feedback reporting days earlier. There was a statistical significance in all the parameters such as initial feedback levels obtained within 15 days ($p = 0.0077$); within 45 days ($p < 0.0001$); above 45 days ($p < 0.0001$); registration status confirmation within 45 days ($p = 0.0343$); mismatch of feedback received by observer ($p < 0.0001$); and telephone number of patients recorded ($p < 0.0001$) (Table 2).

The study conducted in Lahore during January to September 2009 using e-TR as a referral monitoring tool[8] obtained feedback from 45% of patients and in this study, during the first year (2010) the patients' feedback which was not available (wrong address) was 4.5% and after adopting the intervention by sending SMS through mobile phones, it had helped to cut down the no feedback to 2.2% ($n = 184$) and 2.6% ($n = 196$) for the subsequent years of 2011 and 2012, respectively.

In a study[9] from Bangalore at TB Sanatoria, it was observed that use of referral mechanism had shown improvement to 85% of patients reaching for treatment. Similar effect was observed with the intervention during the present study and achieved successful tracing rate (%) was 85:92:89 during the years 2010 to 2012.

The updation of patients' data at field level had resulted in saving time as well as to make communication faster and accurate than the paper-based system in a study[10] done in Tanzania. Similar experience had been observed during this study, facilitating the DTO and STS on tracking the referred patients from SVIMS. The effective role of medical colleges in TB control[5] played in India in providing useful surveillance data and feedback on the programme can be further extended by linking the referred patients data through an online updation in the existing portals by appointing a referral coordinator. The TB/human immunodeficiency virus (HIV) services can be enhanced[11] in this setting and feedback on patients can be communicated to both the programmes.

India stands second in terms of mobile usage[12] and its penetration is very high even in rural areas. This communication can provide an opportunity for RNTCP providers an efficient communication mechanism to transfer message between different stakeholders towards promoting patient adherence and achieving high cure rate. Therefore, in the present study, the effect of using mobile and SMS intervention in reporting patient information into the system; confirmation of TB; information on transferred out patients after referred to TUs; feedback at TU level; confirmation by STS; and tracing of referral subjects have been found to be useful.

After intervention, percentage of subjects reporting feedback had improved; reporting of patient information into the system and confirmation of TB reduced to 30 days from 60 days; communication of registered patient information to DTC had improved; number of subjects transferred outside TU had increased; feedback from majority of TUs improved; averaged days taken to confirm registered patients by STS decreased; and the tracing rate of referral subjects had increased. These results suggest intervention of mobile/SMS can establish cost-effective efficient communication mechanism in the transfer of information between different service providers towards achieving the RNTCP goals for the first time. Additional studies will be crucial for understanding the true benefits and best implementation strategies in RNTCP. These results suggest that SMS reminders may be an important tool to achieve optimal feedback response in resource-limited settings.

In a recent systematic review and meta-analysis[13] that aimed to synthesise current evidence on the effectiveness of SMS interventions in improving TB treatment adherence, it was observed that there was a paucity of high-quality data on the effectiveness of SMS interventions for improving adherence to TB treatment. In the present study, after intervention, we observed that there was a statistically significant increase in all the feedback related parameters such as initial feedback levels obtained; registration status confirmation; telephone number of patients recorded and decrease in mismatch of feedback received by observer. This study is first of its kind to present robust evidence of beneficial effects of mobile phone technology for RNTCP. Additional studies will be crucial for understanding the true benefits and best implementation strategies in RNTCP. These results suggest that SMS reminders may be an important tool to achieve optimal feedback response in resource-limited settings.

The present study was conducted at a tertiary care teaching hospital attached to a medical college in South India. This hospital-based study may be influenced by the referral-bias. Thus, the referral feedback documented in the present study may not necessarily reflect the feedback scenario in the community established in the programme.

Conflicts of interest

The authors have none to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijtb.2016.11.036.

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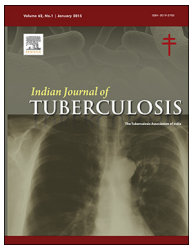

Original Article

Frequency and outcomes of new patients with pulmonary tuberculosis in Hatay province after Syrian civil war

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ABSTRACT

Objective: It is known that tuberculosis is frequently seen among refugees. Hatay province is one of the cities that substantially expose to migration of refugees after Syrian civil war. In this study, it was aimed to compare frequency of new pulmonary tuberculosis (PTB) cases and treatment success/cure rates between Turkish and Syrian patients.

Findings: The study included 211 patients with PTB (178 Turkish and 33 Syrian patients) registered to Hatay Tuberculosis Outpatient Clinic between 2010 and 2013. On the basis of years, number of PTB patients registered was 53 (Turkish/Syrian: 52/1) in 2010, 44 (44/0) in 2011, 41 (39/2) in 2012, and 73 (43/30) in 2013. There were no significant differences between Turkish and Syrian patients regarding age groups, gender, marital status, contact history, smear result, and drug sensitivity assays when treatment success was considered ($p > 0.05$). Directly observed therapy (DOT) rate was higher in patients who achieved successful treatment (97.6% vs. 2.4%; $p < 0.001$). Number of patients successfully treated was smaller among Syrian patients (63.6% vs. 88.8%; $p < 0.001$). Leaving the treatment and/or transfer rates were higher among Syrian patients (30.3% vs. 3.9%; $p < 0.001$). During the study period, drug-resistant tuberculosis was detected in one Syrian and 3 Turkish patients.

Conclusions: Although PTB frequency has increased in Hatay province within prior 4 years, treatment success among local population is still within limits established by World Health Organization (WHO). However, the treatment goal could not be achieved when considered together with refugees. To improve treatment success in refugees, implementation of a new national tuberculosis is needed control program in this population.

1. Introduction

After Syrian civil war, thousands of Syrian citizens immigrated to Turkey as refugees. Refugees first arrived to Turkey in March 2011. There are 200,386 refugees in refugee camps in the supervision of AFAD (Disaster and Emergency Management Presidency) and 350,000 refugees out of these camps.[1] Hatay province is one of the cities which received a substantial number of immigrants since it is the neighbor city to Syria and considerable portion of local community can speak Arabian. Refugees began to settle in Hatay in March 2011. In Hatay

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province, there are 15,400 Syrian refugees in camps and 60,000 refugees residing out of camps by 2013. The population of Hatay province was increased by 5% after migration, which is higher than inherent increase.[1]

During civil wars, TB reactivation and transmission have increased among refugees due to crowded life conditions, immigration, difficulty to access healthcare, nutrition, and shortage of medication and healthcare staff, resulting in increased TB prevalence and mortality.[2–4] In Europe, 19,912 (0.4%) TB cases in Norway and 4643 (0.1%) TB cases in London were detected among refugees.[5,6] TB was the cause of death in 16% of refugees after Somalia civil war while it was the cause of 30–50% of adult deaths in refugee camps in Sudan.[7] TB reactivation is generally seen; however, de novo cases are also encountered within first years after migration.[8,9] In a study using DNA fingerprint in Norway, it was found that refugees were infected prior to migration.[10]

TB prevalence was 23:100,000 in Syria.[11] TB prevalence in Turkey and Hatay province is 26:100,000 and 11.3:100,000, respectively.[12] During Syrian civil war, problems have been experienced in diagnosis, treatment, and prevention of TB due to challenges in access to healthcare services and supply of drugs.[13] In 2013, PTB prevalence has increased to 51:100,000 in Syria.[11]

The most important issue in controlling tuberculosis is early diagnosis and prompt treatment after diagnosis to achieve cure.[14] The World Health Organization (WHO) has established a treatment success rate of 85% in new TB cases.[15]

In Turkey, the diagnosis and treatment of TB is executed by Tuberculosis Outpatient Clinics without any fee. In Hatay, there are two Tuberculosis Outpatient Clinics (1 in Hatay and 1 in İskenderun) and 3 laboratories. In the Tuberculosis Outpatient Clinic of Hatay, TB patients from provincial center and 7 towns of Hatay are being followed. Since 2006, directly observed therapy (DOT) has been employed for treatment of TB in Hatay, which was continued after arrival of Syrian refugees in 2011.

In this study, it was aimed to compare frequency of new pulmonary tuberculosis (PTB) and treatment success/cure rates between Turkish and Syrian patients. This is the first study comparing PTB frequency after the arrival of Syrian refugees.

2. Materials and methods

In Hatay Tuberculosis Outpatient Clinic, 708 patients with pulmonary and extra-PTB had been followed between 2010 and 2013 including 185 cases (74 former and 111 new cases) in 2010, 178 cases (73 + 105) in 2011, 150 cases (65 + 85) in 2012, and 195 cases (62 + 133) in 2013. The study included 211 new PTB patients (178 Turkish and 33 Syrian patients) registered to Hatay Tuberculosis Outpatient Clinic between 2010 and 2013 who completed therapy. This is a retrospective, descriptive study.

Data regarding demographic and disease characteristics were collected by using ''Data sheet for patients with tuberculosis'' which includes 25 questions. By data sheet, demographic characteristics of the patients registered to Tuberculosis Outpatient Clinic (age, gender, marital status, insurance, employment status) and data regarding diagnosis and management disease (presenting complaint, DOT status, history of contact with a PTB patient, degree of relationship with contact, case definition, symptoms, presence of bacille calmette-guerin), scar, status of disease, status of HIV (human immunodeficiency virus) positivity, presence of comorbid disease, result of tuberculin skin test, smear result, tissue diagnosis, results of chest radiography, culture results, results of drug sensitivity tests and outcome) were questioned in the patients. Case definitions include new patient, relapse patient, patients with treatment failure, patients with treatment left, chronic patients, transferred case, and death. New patients are defined as those have never been treated for TB or have taken anti-TB drugs less than one month while relapse patients are defined as those have previously received anti-TB drug one month or more in the past. Patients with treatment failure are defined as those have been previously treated for TB and whose treatment failed at the end of their most recent course while patients with treatment left are defined as those have previously been treated for TB and were declared lost to follow-up at the end of their most recent course of treatment with TB-positive bacteriological studies after withdrawal of therapy beyond 2 months, and the patients who were transferred to another facility to maintain therapy are classified as transferred case.[16] Disease status is classified as PTB and extra-PTB which is defined as TB involving other organs such as pleura, lymph nodes, pericardium, abdomen, joints, bones, and PTB plus extra-PTB. Treatment outcome is classified as cured, treatment completed, treatment failed, treatment defaulted, and died. Outcome is defined according to WHO criteria. Cured is defined as a PTB patient with positive sputum who had 2 negative sputum in addition to clinical and radiological recovery. Treatment completed is defined as a TB patient who completed treatment and had clinical and radiological recovery while treatment failed is defined as a TB patient whose sputum smear or culture is positive at month 5 or later during treatment. Treatment defaulted is defined as a TB patient who did not start treatment or whose treatment was interrupted for 2 consecutive months or more. Died is defined as a TB patient who dies for any reason before starting or during the course of treatment. Multi-drug resistant (MDR) TB is defined as a TB case resistant to at least both isoniazid and rifampicin in drug-sensitivity assays.[16]

2.1. *Statistical analysis*

Statistical analyses were performed by using SPSS version 13 (SPSS Inc., Chicago, IL, USA). Normal distribution of data was assessed by using Shapiro–Wilk test. Continuous variables with normal distribution were compared by using *t* test, while continuous variables with skewed distribution were compared by using Mann–Whitney *U* test. Nominal variables were compared by using Chi-square test. A p value <0.05 was considered as statistically significant.

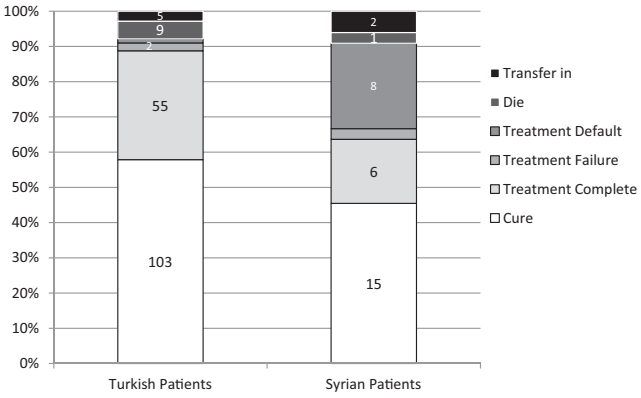
3. Findings

Between 2010 and 2013, 211 patients with PTB (178 Turkish and 33 Syrian) were included to the study. Of these patients, PTB

Table 1 – Distribution of patients with PTB according to years.

Year	Turkish N (%[a])	Syrian N (%[a])	Total N (%[b])
2010	52 (98.1)	1 (0.9)	53 (25.1)
2011	44 (100.0)	–	44 (20.9)
2012	39 (95.1)	2 (4.9)	41 (19.4)
2013	43 (58.9)	30 (41.1)	73 (34.6)
Total	178 (84.4)	33 (15.6)	211 (100.0)

[a] Line percent.
[b] Column percent.



Graph 1 – Outcomes in Turkish and Syrian patients with PTB.

diagnosis was made in 24.3% in 2010, 20.6% in 2011, 18.8% in 2012, and 33.5% in 2013. Highest number of Syrian patients with PTB was noted in 2013 (Table 1). In 2013, there was an increase in the number of PTB cases registered.

Table 2 presents demographic and disease characteristics of Turkish and Syrian patients. Mean age of Syrian patients was significantly lower ($p = 0.001$). PTB was seen in younger age groups among Syrian patients ($p = 0.033$). Male:female ratio was 2:1 and 1.53:1 in Turkish and Syrian patients, respectively.

Of 211 cases, 183 (86.7%) were new cases while there were 8 relapse patients, 4 patients returning treatment failure, 3 patients with treatment left, and 7 transferred patients. Case definition could not be ascertained in 7 cases.

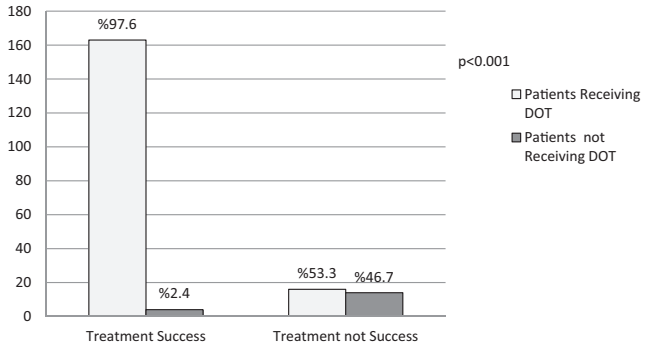
There was no significant difference between Turkish and Syrian patients regarding contact history, presence, and results of smear and drug sensitivity (Table 2). Number of cured patients was smaller among Syrian patients. Loss to follow-up and/or transfer rates were higher among Syrian patients (30.3% vs. 3.9; $p < 0.001$) (Graph 1).

There were no significant differences between Turkish and Syrian patients regarding age groups ($p = 0.574$), gender ($p = 0.201$), marital status ($p = 0.923$), contact history ($p = 0.064$), smear result ($p = 0.638$), and drug sensitivity ($p = 0.441$) when treatment success was considered. DOT rate was higher in patients who achieved successful treatment (97.6% vs. 2.4%; $p < 0.001$) (Graph 2).

Table 2 – Demographic characteristics of registered PTB cases.

	Turkish ($n = 178$)	Syrian ($n = 33$)	p value
Age (mean ± SD)	44.68 ± 19.47	32.27 ± 16.06	0.001[a]
Age groups, *n* (%)			
0–24	30 (71.4)	12 (28.6)	0.003[b]
25–44	56 (80.0)	14 (20.0)	
≥45	92 (92.9)	7 (7.1)	
Gender, *n* (%)			
Male	141 (66.8)	20 (60.6)	0.409[b]
Female	70 (33.2)	13 (39.4)	
Marital status, *n* (%)			
Married	121 (68)	20 (60.6)	0.363[b]
Single	48 (27)	12 (36.4)	
Contact history, *n* (%)			
No	118	24	0.924[b]
Yes	30	5	
Smear test, *n* (%)			
Positive	132 (82.5)	28 (17.5)	0.332[b]
Negative	37 (90.2)	4 (9.8)	
Anti-TB drug sensitivity, *n* (%)			
Sensitive to all agents	74 (80.4)	18 (19.6)	0.589[c]
Resistant to at least one agent	16 (80.0)	4 (20.0)	
Outcome, *n* (%)			
Treatment success	158 (88.8)	21 (63.6)	0.000[b]
Treatment failure	20 (11.2)	12 (36.4)	

"Treatment success" defines "cure + treatment completion".
[a] Mann Whitney *U* test.
[b] Chi-square test.
[c] Fisher exact test.



Graph 2 – Relationship between DOT and treatment success.

MDR TB was detected in one Syrian in 2012 and 3 Turkish patients in 2010, 2011, and 2012, one patient in each year. When all Turkish and Syrian patients were taken into account, there was no significant difference in treatment success between 2010 and 2013 [49 (92.5%), 37 (84.1%), 33 (80.5%) vs. 60 (82.2%); $p = 0.333$].

4. Discussion

It is well known that TB prevalence is higher among refugees.[15] The risk for TB is doubled in this population when compared to general population.[17,18] TB prevalence was 62:100,000 in Iraq before Iran–Iraq war while it was increased up to 74:100,000 after war.[11] In Italy, TB prevalence was 22% in 1999 which reached up to 46.2% in 2006.[17] TB is among frequently seen diseases in the refugee camps in Pakistan after Afghan civil war.[7]

In the present study, we found that PTB was more common in younger age groups among refugees. This could be due to predominance of younger population among refugees in Turkey. Individuals aged 0–18 years comprise more than 50% of all refugees.[1] In previous studies, it was reported that TB was frequent in younger age groups among refugees.[5,6]

Majority of TB cases among Syrian refugees were detected in 2013. Again, majority of all TB cases (Turkish and Syrian) were detected in 2013, which was the year in which most intensive immigration was recorded.[18] In previous studies, it was shown that TB incidence was high among refugees but this caused minimal increase in TB incidence in places where they settled.[19,20] In 2013, TB prevalence was found to be 36:100,000 among refugees after Syrian civil war in Jordan and this rate was twofold higher than TB prevalence in Jordan.[21] The higher prevalence of TB among refugees could be due to crowded life conditions and malnutrition,[7] and physiological and psychological stress.[22] In another study, unemployment, alcohol, substance abuse, and contact with index case were found as independent risk factors for increased TB cases among refugees.[23] In a study on Tibetan refugees, it was found that crowded life conditions and high prevalence of latent TB infection were associated to increased TB prevalence.[18] Active screening can be recommended to decreased TB prevalence.[24] In Canada where refugees frequently seek asylum, prophylaxis was given against latent TB infection. In addition, individuals with latent TB infection and those with sequel on chest radiography were followed by surveillance programs one month after settlement.[25] American Thorax Society recommends to screen all refugees by tuberculin skin test or interferon gamma release test in order to reduce TB prevalence.[26] Similar practices are also employed in Italy.[17] A TB control program based on case identification and review of therapeutic regimens can be implemented in order to reduce TB prevalence among refugees.[21] In our study, treatment success was lower in Syrian patients. This could be explained by higher rates of loss to follow-up and transfer rates as well as lower DOT rates. In previous studies, it was found that factors such as advanced age, male gender, MRD TB, diabetes mellitus, poor socioeconomic status, difficulty in accessing healthcare services, incompliance to treatment, lack of sufficient information about disease and its treatment, and insufficient social support were associated to treatment failure.[27–29] In a study from Turkey, it was found that treatment failure and drug resistance were associated to advanced age, being born abroad, bilateral radiological involvement, and presence of cavitary lesions, relapse, and incompliance to treatment.[30] In Jordan, the project 'Public Health Strategy for Tuberculosis among Syrian Refugees in Jordan' was executed between 2012 and 2013 in order to improve treatment success among refugees. By implementation of this project, case screening studies were enhanced among Syrian refugees and diagnostic procedures were performed meticulously. DOT was employed to improve treatment success, and education materials and brochures were provided to individuals in camps and those out of camps in order to increase awareness of TB. National measures were supported for management of latent TB. At the end of project, goal for cure rate (85%) in new cases established by WHO was achieved with cure rate of 91%.[21]

In our country, rate of MDR TB cases was found to be 2.7% in 2011 [12]. MDR TB is of importance in clinical manner due to poorer prognosis, low cure rates, and greater treatment costs.[31,32] In our study, MDR TB case was detected in 3 Turkish patients in 2010, 2011, and 2012, as being one patient in each year. In addition, MDR TB was detected in one Syrian refugee in 2012. MDR TB is an important issue in refugees. MDR TB prevalence is rather high in Eastern Europe countries, Southeastern Asian countries, Dominic Republic, Leetonia, and some former Soviet countries.[31–33] Immigration from these regions to developed countries such as USA, Canada, Western Europe, and Australia makes this issue a global problem requiring international approaches. In the present study, no significant difference was detected between 2010 and 2013 regarding treatment success when all patients included to the study were assessed. This could be due to smaller number of refugees among these years and meticulous execution of DOT in Hatay province.

Although TB frequency has increased in Hatay province after arrival of refugees, treatment success among local population is still within limits established by DOT. However, treatment goal could not be achieved among refugees. To improve treatment success in refugees, implementation of a new national tuberculosis control program is needed in this population.

Conflicts of interest

The authors have none to declare.

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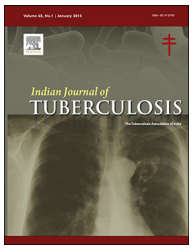

Original Article

Resident doctors' attitudes toward tuberculosis patients

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ABSTRACT

Background: The attitude of the resident doctors toward tuberculosis (TB) patients can affect their treatment seeking behavior, compliance to treatment as well as reinforce the stigma attached to the disease by the society at large.
Aims: To assess the attitudes of resident medical doctors toward TB patients.
Material and methods: A cross-sectional study was conducted among postgraduate resident medical doctors at B.J. Government Medical College and Sassoon General Hospital, Pune in September 2014. The background characteristics and attitudes were assessed using a semi-structured questionnaire. The responses were analyzed using Chi-square/Fishers exact test and calculating odds ratio (OR).
Results: Of the 212 resident doctors who responded to the question on attitudes, 132 (62%) see TB patients on a daily basis, 40 (19%) of the resident doctors had attended a training program on TB, and 99 (47%) respondents knew of a colleague with TB. Only 104 (49%) of the residents reported feeling compassion for and the desire to help TB patients. The residents who had attended a training program in TB were three times more likely to report compassion and a desire to help TB patients than those who had not undergone such training [28/40 vs 76/172; $p = 0.005$; OR = 2.95, 95% CI (1.33–6.61)]. Compared to residents who did not know of a colleague with TB, residents who knew of a colleague with TB were nearly three times more likely to avoid managing TB patients or fear them and think they may cause infection [33/99 vs 17/113; $p = 0.002$; OR = 2.82, 95% CI (1.39–5.76)].
Conclusion: The feeling of fear, lack of compassion, and tendency to avoid TB patients reported by 51% of the patients is a cause of concern. Addressing the knowledge gaps through training programs and ensuring safe working environment will make residents more supportive and compassionate toward TB patients which will contribute to TB control.

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1. Introduction

One of the ways by which activities of health professionals may expose patients of tuberculosis (TB) to stigmatization is their behavior toward patients with TB.[1] This may reinforce the stigma attached to the disease by the society at large which has wider socioeconomic ramifications. The feelings of Health Care Workers (HCWs) toward TB patients may have an impact on their own behaviors when they manage the TB patients.

Treatment of TB patients with the Directly Observed Treatment-Short course therapy under Revised National Tuberculosis Control Program (RNTCP) is peculiar as patients are expected to visit the health units at regular intervals and have frequent encounters with the health care workers. All TB patients need to be compliant with their treatment to get well and their interaction with health care providers can be a key factor in how compliant patients are with their care. Studies have described that the attitudes and behavior of health care workers affects the health care seeking behavior leading to delay in diagnosis as well as impairs adherence to treatment.[2,3] Patients not treated well by the health care system or stigmatized are less likely to take their medications and be cured, increasing the risk of TB mortality and Multidrug-Resistant TB (MDR-TB) as well as continuing the chain of transmission in the community.

Resident doctors are medical graduates who have registered for post-graduation courses at teaching hospitals and play a key role in the management of patients, particularly during the initial diagnosis phase and whenever complications arise. They are key providers of diagnosis and treatment to TB patients, particularly the sickest TB patients who need hospitalization in a tertiary health care center. We do not have much information on the attitudes of resident doctors toward TB patients. We undertook this study to document their attitudes and identify opportunities to improve the care of TB patients and reduce the stigma they feel.

2. Methodology

A cross-sectional study was conducted at B.J. Government Medical College and Sassoon General Hospital, a government teaching hospital in Pune, India in September 2014. The study population included post-graduate medical resident doctors of clinical and laboratory based para-clinical departments.

A pre-tested semi-structured questionnaire was used for data collection. The question for assessing TB attitudes and stigma suggested in the World Health Organization (WHO) guide for knowledge, attitude, and practice surveys was modified to assess attitudes toward TB patients.[4] The question enquired their feeling about patients with TB disease with the following options: (a) ''I feel compassion and desire to help.''; (b) ''I feel compassion but I tend to avoid managing such patients.''; (c) ''I fear them because they may infect me.''; (d) ''I have no particular feeling''; and (e) Others. The responses in the 'others' category were reclassified into the four options for further analysis.

The questionnaire included questions on other variables, including residence, duration of work, frequency of exposure to TB patients, past history of TB, training on TB and knowledge about another resident with TB.

This study was reviewed and approved by the ethics committees of B.J. Government Medical College and The Johns Hopkins University School of Medicine. Written informed consent was obtained from each respondent. Investigators distributed the questionnaires to the residents either at the beginning or end of a meeting as part of standard departmental postgraduate academic activities.

The associations between background characteristics and attitude were assessed using Chi-square/Fisher's exact test using SPSS (Version 16). A p-value of less than 0.05 was considered to be statistically significant. Odds ratio (OR) with 95% confidence interval were calculated to assess the strength of association.

3. Results

Of 325 resident doctors registered for postgraduate courses at the institute, 305 (94%) were invited to participate. Of the 20 resident doctors who could not be contacted, seven were on leave and 13 were posted on rotation to another department. Out of the 263 respondents (86% of residents contacted) who consented to participate, 212 (81%) responded to the question on the feeling about patients with TB disease.

Of the 212 respondents, 116 (55%) were males, 174 (82%) were residing in a hostel, 108 (51%) reported to work for 12 h or more, and 132 (62%) had daily exposure to TB patients. Only 40 (19%) reported to have attended a training program on TB in the past. A total of 35 (17%) were assessed for TB in the past while 10 (5%) had a past history of TB. A total of 99 (47%) residents knew of a colleague who had been diagnosed with TB and 59 (28%) knew of a colleague in their own department who had TB.

Only 104 (49%) residents reported feeling compassion and a desire to help TB patients. The rest reported to feel compassion yet avoid TB patients, fear and think that they may cause infection or have no particular feeling. (Table 1) The comments mentioned in the 'others' category of responses include – *'Whatever anyone may feel we have to manage patients in wards' (Category: Have no particular feeling); 'TB is treatable and would want to treat, manage and cure them' (Category: Feeling compassion and a desire to help); I feel compassion and desire to help but after ensuring my safety' (Category: Feeling compassion and a desire to help).*

Having attended a training program in TB was significantly associated with their attitude toward TB patients ($\chi^2 = 13.54$; df = 1; $p = 0.004$) (Table 2). The residents who had attended a training program in TB were three times more likely to report compassion and a desire to help TB patients than those who

Table 1 – Attitude of resident doctors toward tuberculosis patients.

Feeling about patients with TB disease	N	%
I feel compassion and desire to help	104	49.06
I feel compassion but tend to avoid managing such patients	23	10.85
I fear them and think they may infect me	27	12.74
I have no particular feelings	58	27.36
Total	212	100.00

Table 2 – Attitude of residents toward TB patients according to background characteristics.

Variable	Groups	Compassion and desire to help N = 104	Compassion but tend to avoid managing such patients N = 23	Fear them and think they may infect me N = 27	No particular feelings N = 58	Chi-square	p
Gender	Male (N = 116)	58 (50)	12 (10.30)	12 (10.30)	34 (29.30)	0.016	0.66
	Female (N = 96)	46 (47.90)	11 (11.50)	15 (15.60)	24 (25.00)		
Year of residency	First year (N = 56)	27 (48.2)	8 (14.3)	7 (12.5)	14 (25)	0.02	0.9
	Second year (83)	40 (48.2)	8 (9.6)	9 (10.8)	26 (31.3)		
	Third year (73)	37 (50.7)	7 (9.6)	11 (15.1)	18 (24.7)		
Residence	Hostel (174)	86 (49.4)	20 (11.5)	20 (11.5)	48 (27.6)	0.016	0.66
	Local residence (38)	18 (47.4)	3 (7.9)	7 (18.4)	10 (26.3)		
Duration of work	<12 h (104)	56 (53.8)	10 (9.6)	15 (14.4)	23 (22.1)	3.75	0.29
	≥12 h (108)	48 (44.4)	13 (12)	12 (11.1)	35 (32.4)		
Frequency of exposure	Every day (N = 132)	59 (44.7)	15 (11.4)	22 (16.7)	36 (27.3)	5.68	0.128
	Others (80)	45 (56.2)	8 (10)	5 (6.2)	22 (27.5)		
Attended training program on TB	No (N = 172)	76 (44.2)	18 (10.5)	22 (12.8)	56 (32.6)	13.54	0.004
	Yes (N = 40)	28 (70)	5 (12.5)	5 (12.5)	2 (5)		
Assessed for TB in past	No (N = 177)	83 (46.9)	18 (10.2)	23 (13)	53 (29.9)	0.042	0.245
	Yes (N = 35)	21 (60)	5 (14.3)	4 (11.4)	5 (14.3)		
Past H/o TB	No (N = 202)	99 (49)	20 (9.9)	25 (12.4)	58 (28.7)	6.86	0.07
	Yes (N = 10)	5 (50)	3 (30)	2 (20)	0 (0)		
Know of a colleague with TB	No (N = 113)	58 (51.3)	7 (6.2)	10 (8.8)	38 (33.6)	11.3	0.01
	Yes (N = 99)	46 (46.5)	16 (16.2)	17 (17.2)	20 (20.2)		

had not undergone such training [28/40 vs 76/172; $p = 0.005$; OR = 2.95, 95% CI (1.33–6.61)].

Knowing another colleague who had been diagnosed with TB was significantly associated with their attitude toward TB patients ($\chi^2 = 11.3$; df = 1; $p = 0.01$) (Table 2). Compared to residents who did not know of a colleague with TB, residents who knew of a colleague with TB were nearly three times more likely to report a tendency of avoiding TB patients or fear them thinking they may cause infection [33/99 vs 17/113; $p = 0.002$; OR = 2.82, 95% CI (1.39–5.76)].

4. Discussion

Only 49% of the resident doctors surveyed reported compassion and a desire to help TB patients. Studies have reported instances in which the service providers have been rude, unhelpful, and did not provide attention and support to TB patients.[5,6] On the other hand, a cordial relationship between patients and health staff has been shown to be the main motivating factor for completion of TB treatment.[7]

Resident doctors who reported prior TB-specific training were more likely to have a positive attitude toward TB patients. But very few resident doctors reported to have attended such training. In a prior study, training workshops in TB control were demonstrated to be effective for promotion of knowledge and elimination of stigmatization in first-line caregivers.[8] Our data supports the need and value of providing all resident doctors caring for TB patients with specific training.

Residents with knowledge of a colleague with TB may perceive the risk of occupationally acquired TB to be more real and hence report feeling afraid of TB patients and an unwillingness to help. Studies from India have reported the risk of occupationally acquired TB among health care workers along with inadequate infection control measures in place.[9] Hence, it is necessary to address the issues regarding prevention and control of transmission of TB in health care setup. It is important to ensure occupational safety for health care workers with a safe working environment and adequate measures for prevention of TB transmission at workplace. In addition, well-defined strategies to minimize TB-related stigma and discrimination can be utilized to formulate and implement sustainable TB anti stigma campaigns.

This study throws light on the attitude of resident doctors at a tertiary health care center though the findings cannot be generalized to other cadres on health care workers at the institute or working under RNTCP. Yet it raises the issue of the feeling of fear and apprehension about TB patients among health care workers which needs to be studied and addressed urgently.

While we assume residents have a moral responsibility to be supportive and compassionate toward their patients, addressing knowledge gaps and ensuring safe workplace may enhance natural tendencies that have been suppressed due to stigma and fear. In addition, trainees are in a unique position to contribute to de-stigmatize TB in their community, through their attitudes and behavior toward TB patients.

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Authors' contribution

GP contributed to conception and design, acquisition of data, analysis and interpretation of data, drafting the manuscript;

DK, AC, RB and AD contributed to conception and design, revising it critically for important intellectual content and final approval of the version to be published.

Conflicts of interest

The authors have none to declare.

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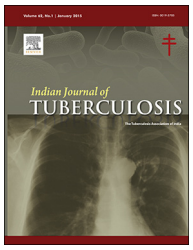

Original Article

Patients' perception towards directly observed treatment – A qualitative study from Kollam district, Kerala, southern India

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ABSTRACT

Background: The Direct Observation of Treatment (DOT) is an important component of the country's TB Control strategy. Standards of TB care in India and the End TB strategy emphasised the importance of a patient-centered approach to foster adherence. A qualitative study was conducted to explore the perception of people with Tuberculosis in Kerala regarding DOT, mechanisms to make the treatment of TB more patients centered and to identify the preferable mechanisms to ensure adherence.

Methods: Six focus group discussions were conducted – two among people with TB from rural area, two among people with TB in urban area, one among multipurpose health workers of rural area and one among key field staff of TB control in urban area.

Results: Patients who were on a strict DOT were unhappy about the issues of *confidentiality, patient inconvenience and provider centered approach*. A flexible, patient centered approach were a family member can act as the DOT provider with guidance from a trained health worker was evolved as the most acceptable and comfortable mode of treatment to majority of the TB patients. They felt that a strict external monitor as a DOT provider was not a necessity in majority of the cases. Only practical way to effectively incorporate ICT in monitoring patient compliance in current scenario was identified as daily phone call reminders. Patients also expressed their concerns in keeping the medicines for entire duration at home.

Conclusion: A flexible patient wise individualized system based on patient's behavior, literacy and awareness along with attitude of family members is needed to ensure adherence to anti TB drugs.

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1. Background

Revised National Tuberculosis Control Program (RNTCP) in India is shifting from intermittent regimen to daily fixed dose combinations for treating drug sensitive TB. The principle of treatment for TB henceforth will be to administer daily fixed dose combinations of first line anti-TB drugs in appropriate weight bands. Kerala is identified as one among the five States implementing daily regimen in first phase in India.

Adherence to regular and complete treatment is one of the important factors for relapse-free cure from TB.[1] Treatment adherence is also a critical determinant of treatment outcomes, prognosis and further emergence of drug resistance.[1–3] The Directly Observed Short course Strategy (DOTS) has been the backbone of country's TB programs for the last two decades.[4] The Direct Observation of Treatment (DOT) is an important component of the strategy and is an attempt to improve adherence by active monitoring and recording of the consumption of each and every drug dose by an 'observer' acceptable to the patient and the health system.

Some systematic reviews challenged the dogma that DOT improved cure in TB.[5] Also, there have been opinions that DOT is a coercive model which leaves the patient only as a passive recipient of therapy. There are also debates as to the best delivery of DOT, for example, should it be through healthcare workers or family members. Literature shows that there are varied perceptions of DOTS among TB patients and providers.[6]

Standards of TB care in India (STCI) and the End TB strategy emphasized the importance of a patient-centered approach to foster adherence. It has also been laid down in STCI that treatment adherence goes beyond the realm of DOTS.[7] Many Information Communication Technology (ICT) based models has also been piloted in the country for ensuring adherence with success.

On this background, we conducted a qualitative study to explore the perception of people with Tuberculosis regarding DOT, how the treatment of TB could be made more patient-centered and effective and preferable mechanisms to ensure adherence. Perceptions of health workers experienced in TB control were also captured. The results of this study would enrich the understanding of DOT from patient's perspective and help the policy makers and the program managers to tailor the treatment support mechanism to ensure adherence in Kerala State.

2. Methods

Kollam district has a population of 2.6 million and represents a typical mix of urban and rural population. Literacy rate of females is 92%. Kollam district reflects the typical state scenario in terms of geography and health care delivery. The district examined 278 per lakh TB suspects and notified 38 per lakh new smear-positive TB cases in 2014.

A total of six focus group discussions (FGD) were conducted two among people with TB from rural area, two among people with TB in urban area, one among multipurpose health workers of rural area and one among key field staff of TB control in urban area.

A FGD guide was developed and the key themes of the FGDs were (a) What is your view about DOT? (b) What do you think from your personal experience are the advantages and disadvantages of DOT? (c) What is your view about Family member acting as DOT provider? (d) What do you think should be the role of an external person or health worker in helping you? (e) How, from where, from whom, and how frequently do you prefer to get anti-TB medicines for your treatment? (f) What do you think are the reasons for non adherence to anti-TB medications? (g) What are the factors which help a person to complete his anti-TB treatment without fail? and (h) What do you think is the role of Information, Communication, Technology to help a TB patient with his/her treatment.

Multipurpose workers identified adult TB patients registered in RNTCP who had either just completed their treatment (within one month) or are in the last month of treatment and were willing to share information as participants. FGDs were conducted at the conference halls of nearest primary health centers or training centers without interference from outside. Even health workers were not allowed inside the hall. Wives of two of the patients accompanied them were allowed to hear the discussion.

The aims of the investigations and implication for participation were explained at the start of the FGDs. What is ideal DOT was also explained in the beginning. Confidentiality was ensured and participants were given a chance to opt out freely at that stage without giving any reason. Demographic details were also collected from the participants. All

Table 1 – Socio-demographic characteristics of the study participants.

Characteristics	Categories	Rural area N = 14	Urban area N = 15
Age	15–30 years	1 (7.1%)	1 (6.7%)
	31–45 years	3 (21.4%)	4 (26.7%)
	46–60 years	6 (42.8%)	7 (46.7%)
	More than 60 years	4 (28.5%)	3 (20%)
Gender	Male	9 (64.2%)	10 (66.7%)
	Female	5 (35.7%)	5 (33.3%)
Socio economic status	BPL	11 (78.6%)	9 (60%)
	APL	3 (21.4%)	6 (40%)
Educational status	Illiterate	2 (14.2%)	1 (6.7%)
	Upto 4th standard	4 (28.5%)	2 (13.3%)
	5–9th standard	3 (21.4%)	3 (20%)
	10 standard pass	3 (21.4%)	3 (20%)
	10–12th standard	2 (14.2%)	3 (20%)
	Graduate/post graduate	0	3 (20%)
Occupation	Not going for work	4 (28.5%)	3 (20%)
	Household activities	4 (28.5%)	3 (20%)
	Student	1 (7.1%)	1 (6.7%)
	Unskilled/semiskilled	5 (35.7%)	3 (20%)
	Skilled work/petty business	0	2 (13.3%)
	Clerical/Office work	0	1 (6.7%)
	Semi/Professional	0	2 (13.3%)

the FGDs were moderated by the same person who was experienced in conducting FGDs and was fluent in the local language. The moderator ensured that the themes were fully discussed and that all participants were given a chance to express their views fully. Each FGD lasted for 40–60 min with additional 10–15 min for informal conversations. The proceedings were audiotaped with the consent of participants. Two researchers recorded the proceedings, noting key themes and monitoring verbal and non-verbal interactions.

The audiotapes were transcribed verbatim. These were in Malayalam and were translated into English before coding. Themes were divided into View regarding DOT, perceived advantages and disadvantages of DOT, need of an external support, concept of Family DOT, causes and suggestions to reduce default, and role of ICT in monitoring compliance. The team read the transcripts and notes and reached a consensus. Any disagreements were discussed regularly within the team to reach a consensus regarding theme coding. Sections with similar coding were grouped according to the predetermined themes. Repeated themes were marked as important in red font color. All the flagged statements were put together and synthesized. Important quotations were quoted which evoked spontaneous discussion, around which a lot of time was spent and had some emotional cues attached with.

3. Results

Twenty-nine people who had just completed the TB treatment and eight health/TB workers participated in FGDs. The demographic characteristics of the 29 people who had TB were given in Table 1.

4. View about DOT

We felt that already a 'patient friendly and flexible' approach is being followed in rural areas and so itself patients were happy about the system. Most of the DOT providers used to hand over the drugs to patients at patient's home. Many times due to the 'trust' with the patient DOT provider may not wait till the patient swallows the tablets. There are DOT providers who used to 'deliver' the medicines at 6 am in the morning considering patient convenience. There are incidences where the provider has issued more than one dose even in intensive phase and medicines handed over regularly to a relative as the patient is old and finds it difficult to reach the center. But when asked about their view about ideal DOT scenario, many of them reported it as inconvenient.

In urban areas, we realized that a stricter DOT is followed. Even though they agree that it helps in ensuring adherence and chance of missing doses, all of them reported DOT as inconvenient to them. Some of them preferred to take DOT from a distant place for fear of losing confidentiality. They are of the opinion that community volunteers are not good enough to keep confidentiality. Two of them commented that they know people who go to private hospital for getting medications only because of the inconvenience due to DOT. Many felt that DOT has affected their work and affected their travel to attend social functions.

A 24 year old girl with lymph node TB said ''*I had no problems at all going for work. But because of the fact that I need to come here on alternate days, I left the job. I was offered treatment from a DOT provider nearby, but I denied due to confidentiality issues*.''

A 60 year old educated man said ''*there were instances when I couldn't come and take medicines from here because of emergencies, family functions etc. But still I tried my best not to miss the doses*.''

5. Factors associated with satisfaction of DOT

It was evident that DOT provider acceptability is an important factor associated with satisfaction toward DOT. Generally they appreciated friendly DOT providers who understand the patient circumstances, who respect patient confidentiality, who go 'out of the way' by issuing drugs at patient's home, who is ''flexible'' by giving more than one dose and even to patient relatives. But the general feeling was that community DOT providers like ASHAs are poor in maintaining patient confidentiality. Patients who preferred distant centers, even though inconvenient to them, did it only for fear of confidentiality.

A 50 year old man from urban setting quoted -''*TB has a social stigma. Bitter experiences had been there even from heath workers at DOT centers. This increases our mental stress and hurts us badly*''

6. Family DOT

All participants from rural and urban area agreed to the idea of family DOT. All groups opined that family members are most concerned with cure and are the ideal people to provide medicines. They reported that they would have been more comfortable when given by close relatives.

A 58 year old man in rural setting said ''*getting medicines from wife/dear ones are more satisfactory and comforting to mind as compared to getting it from a stranger/outsider*''

A 64 year old man in urban area said 64 year old man from urban setting told that ''*I am coming to hospital and taking medicine regularly as my daughter is insisting me to do so. So even if I am given my medicines home, I will regularly take the medicines as my daughter constantly reminds me*''

At the same time, they felt that for some people family DOT will not work. All groups raised the issue of chronic male alcoholics as an example. There was a 42-year-old chronic alcoholic person in the FGD in rural area who actually agreed to the idea of family DOT. But his wife, during the informal conversations, said that it would not have been possible by her to manage his treatment alone. Also, for those without adequate family support, family DOT would be difficult.

A 64 year old man in rural area said that "*I don't have anybody in home throughout to look after and give medicines. So I need someone to remind me and provide medicines*"

7. How, from where, and how frequently do you prefer to get anti-TB medicines for your treatment?

Even though they all agreed to the concept of Family DOT, majority were against the idea of keeping the box of medicines for the entire duration at home. Only a very few (3/14) from urban area agreed to the idea of keeping medicines for the entire duration at home. Mental stress on seeing the bulk of tablets and confidentiality issues of keeping medicines in boxes were highlighted as the main reasons for not preferring to keep the box of TB medicines at home. Lack of safe area at home for keeping medicines and the remote possibility of suicidal attempts were also raised as concerns.

A 40 year old lady from urban area commented that "*seeing this whole lot of drugs when given to home will create a mental stress and this itself may be a reason for many not to take drugs*"

A 55 year old lady at rural area said "*many people including some relatives and neighbors may visit house. They will start asking what the medicine inside this box is for ?*"

A 58 year old man from rural setting said that "*A TB patient is already under mental stress and in addition if there are family problems and financial burden, the sight of these many drugs inside a box at home may tempt him to take all the drugs at once and commit suicide*"

People at urban areas reached a consensus that they need medicines for one-month duration at a time. Both groups in rural area were comfortable if they get the medicines for at least for one week.

People at rural area are happy to get the medicines for longer duration from their current DOT providers or Primary Health Centers while urban area people would like to get it from Primary Health Centers or District TB Center, but not from community DOT provider.

8. Need of support from an external person or health worker

People at rural areas unanimously opined that they need support from health workers for curing their illness while people at urban areas had a mixed opinion.

We felt that the patients were well aware about the illness, duration of treatment and the need to complete it. They agreed that the education by the health workers – Multi-purpose health workers in rural areas and TB Health visitors in urban areas have definitely helped them in understanding the disease. Many even said that the health workers helped them by providing psychological support during initial periods of illness. To know about how to administer drugs and to clarify doubts as and when required everybody agreed that they need some form of support. However, most of them did not prefer health workers to visit their houses as recurrent visits may hamper confidentiality. They are comfortable in meeting health workers frequently at health centers and to keep in touch over phone. They prefer to get in touch with health workers or knowledgeable persons rather than community volunteers.

A 34 year old lady emphasized the need of an external support by telling "*when I felt nausea I used to call sister (female health worker) and it is a mental relief when she consoles me by giving health educations and measures to be taken if needed*"

Another 68 year old man from rural are said that "*I was a chronic alcoholic and smoker. After diagnosed with TB, health workers recurrently visited my home and gave health education. Finally I stopped alcoholism and smoking and now my condition drastically improved*"

9. Reasons for interruption and solutions

Forgetfulness was the major reason raised by the groups for treatment interruptions. But the interruptions would not be for more than a day or so. They all agreed that having a family member to remind is the one of the best options. Majority of them attributed their major reason for their completion as the care and support of the family. But there are isolated instances when the family member also forgets about the medications.

Getting aware and self-motivation were suggested as the best options to complete treatment. Education and advises from health workers have helped them to know about the illness and motivates them to complete treatment.

Chronic alcoholism was the major reason cited by the participants for interrupting treatment. However, they could not suggest a recommendation for dealing with the same. They said that alcoholics are not interested in health and always urge to take alcohol.

"*He (problem alcoholic) may not take even if it is community DOT, institutional DOT or family DOT*" – a 64 year old male in rural area

Side effects of the anti-TB drugs were also cited as reasons for interruptions. Many among the group had purposefully skipped doses due to side effects like itching and vomiting. However, consolation and counseling by health workers have helped them to overcome the problem.

10. Use of ICT in TB treatment

Majority of them in rural areas, especially old members, did not know how to use a short message service in mobile phones. A few of them did not even have mobile phones of

their own; however, every house has mobile phones. They said that they would be comfortable if they receive phone calls regularly enquiring about their illness and reminding about the drugs. When asked them about the possibility of sending missing calls to numbers after taking drugs, people at rural areas were not that much comfortable with the idea. They said that they prefer the provider initiated calls which would also help them to remind about the drugs. They also expressed their comfort in receiving health education messages over phone. They prefer to have facilities for clarifying doubts on as and when needed basis. People at urban areas are a little more comfortable with ICT use, but there also older people were not used to sending test messages.

A 58-year-old man from urban setting quoted

''*Cheaters may cheat. So methods like missed call feedbacks, relying on returning empty blister packets etc. may not be effective in helping them.*''

11. FGD with health workers and TB control workers

All health workers from urban area favored the opinion of Family-DOT provided that they have a supportive and caring family.

''*Most of our patient find difficulty with community DOT for fear of confidentiality and institutional DOT due to inconveniences in their day to day life. Many are educated and have good family support. We can blindly follow family DOT in such cases*'' – a female TB HV in urban area.

''*The current timing of medicines in morning hours is inconvenient to most of the patients. It will interfere with their work especially due to fatigue following drug intake. In Family DOT timing of the drug intake can be adjusted according to the patient's convenience*'' – a multipurpose worker in urban area

FGD in urban area opined that 6/10 TB patients will be eligible for a family DOT, the group working in rural area told that only 3–4 out of 10 patients may complete treatment with Family DOT alone. Health workers in rural area emphasized the necessity of supervised administration at least on weekly basis as majority of the patients may not adhere to full treatment if whole drugs are given to them. They also agreed the need to build the capacity of community DOT provider to maintain confidentiality.

''*Family DOT does not mean that we can sit quietly. Our workload will increase. There should be some system for identifying interruptions*'' said a female worker at urban area

All of them were of the opinion that a strong supportive system with intensive counseling and monitoring especially during the initial stages by trained health professionals is needed and is the best determinant of patient completing the treatment.

''*. . .and counseling and education cannot be complete in one sitting. To be effective it may need multiple contacts with the patient*'' – said a female TB control worker

TB control workers were of the opinion that they have the capacity to identify the probable defaulters in the first sitting itself, the multipurpose health workers in rural area said they need one month observation of the patient to make such a decision.

''*. . .for those who have a tendency to default, strict DOT has to be ensured*'' – TB control worker

Health workers from urban area had the opinion that phone call monitoring is better and is a useful measure. Automated programmed calling system is effective as it helps in maintaining confidentiality and a few working in urban area felt that close relatives should be called to enquire about interruptions instead of calling the patients.

The group reached a consensus that one strategy will not fit into all patients. Many could be left to family after initial assessment with a support mechanism for counseling and education. They should be asked to visit health centers on a frequent basis during initial period. The family DOT with support and monitoring will take care of 70–80% of the cases. Other difficult to tackle people, especially alcoholics and those without family support, should be dealt through current DOT strategy. The workers are of the opinion that involving local political and community leaders should be resorted only as the last option for want of confidentiality issues.

12. Discussion

''Patient-centered approaches'' mean interventions that focus on sharing decisions about the management of health problems with patients and that view the patient as a whole person who has individual preferences situated within a wider social context. From the study findings, it is felt that in rural area, people are comfortable with the DOT practiced, which is *flexible, patient friendly, and home-based*. In contrast, people from urban area who were on a strict DOT were unhappy about the issues of *confidentiality, patient inconvenience and provider-centered approach*.

The study is done among TB patients in Kollam district and cannot be generalized to settings outside the State. There could be a selection bias in this study as health workers selected the patients and only those who are 'friendly' with the health workers would have turned up. FGDs provide general perception and do not provide information at individual level. The strength of the study is a set of well-conducted FGDs focusing on patient's and health worker's perspective.

A flexible, patient-centered approach where a family member can act as the DOT provider with guidance from a trained health worker is the most acceptable and comfortable mode of treatment to majority of the TB patients. Family-DOT may not ensure compliance in patients who tend to default, especially alcoholics, and also it is a limitation in an unsupportive family members or lack of affectionate relatives. A flexible patient-wise individualized system based on

patient's behavior, literacy, and awareness along with attitude of family members will be more effective. A strict external monitor as a DOT provider is not a necessity in majority of the cases. Recurrent home visits by health workers were not encouraged as it raises an issue of confidentiality and create a social stigma.

But a trained health worker who is accessible and approachable to patients is preferred by the patients to create awareness, provide psychological support, and also to answer their queries. In an area where TB is clustered more among the older age groups, the only practical way to effectively incorporate ICT in monitoring patient compliance is daily *phone call reminders and intermittent phone call enquiries and feedback* from the patient as well as relatives. Could ICT-based phone call mechanisms minimize some of the responsibilities of health workers like awareness generation and answering to queries need to be explored further. Poor ICT literacy is challenging for other novel strategies in the current scenario. Patients have concerns in keeping the medicines for entire duration at home. Medicines for one-month duration from institutions were preferred.

It important that policy makers, practitioners, and patient support groups acknowledge the patient autonomy in the treatment process; the importance of patient-centered interventions that encourage shared decision-making regarding treatment; the role of support systems tailored to patient needs; the role of informal, societal structures in reinforcing adherence through patient support. Capacity building of the health workers is a necessity to make them organize tailored support system to patient's needs focusing more on the structural and societal factors. The findings also direct to the need of an experimental study to assess the effectiveness of family DOT in Kerala.

Conflict of interest

The authors have none to declare.

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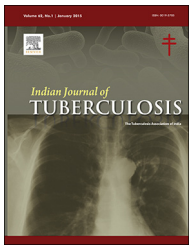

Original Article

Conventional TBNA experience over a 10-year period: Diagnostic yield and associated limitations in a tertiary care government set-up

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ABSTRACT

Introduction: It is challenging for pulmonologists to sample mediastinal lymph nodes or some endobronchial lesions because of safety concerns. C-TBNA (conventional transbronchial needle aspiration) is a procedure to sample such sites, but is underutilized. We present a retrospective review of patients subjected to C-TBNA through fibre-optic bronchoscopy over a 10-year period.

Materials and methods: Year-wise statistics of C-TBNA was reviewed and results were analyzed with regard to sampling sites and type of intraluminal lesions encountered, diagnosis made and their correlation with sampling sites, sex and age.

Results: 160 patients underwent successful C-TBNA with 111/160 (69.4%) males and 49/160 (30.6%) females. Non-availability of in-house needles dramatically decreased the number of procedures.

17 (10.6%) patients underwent C-TBNA from intraluminal bulge, 41 (25.6%) from endobronchial growth and 102 (63.8%) from enlarged lymph nodes. Subcarinal lymph node alone was predominantly aspirated in 83/102 (81.4%) lymph node sampled patients. In 100 (62.5%) patients, diagnosis was achieved as follows: 57/100 as tumour, 30/100 as infection and 13/100 as sarcoidosis. Non-small cell lung cancer (NSCLC) and tuberculosis (TB) predominated in tumour and infection groups, respectively. Patients with intraluminal growth or bulge had higher chances of being diagnosed with tumour ($p < 0.001$). Intraluminal bulge and growth predominated in older ages while enlarged lymph nodes predominated in the young ($p = 0.018$). Infection was predominantly diagnosed in younger patients, sarcoidosis in the middle aged, and tumour in older patients ($p < 0.001$).

Conclusion: C-TBNA should be used as a diagnostic tool in developing countries like India. It can give confirmatory results in difficult cases with intraluminal growths and submucosal lesions. Cost constraints are of paramount importance, and hence continuous supply of expensive accessories should be ensured.

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1. Introduction

Numerous diseases present with mediastinal lymph node enlargements or endobronchial lesions where diagnosis is challenging because of difficulties in accessing the sampling sites and associated safety concerns. Conventional transbronchial needle aspiration (C-TBNA) is a method to sample lesions in these 'difficult to access sites' through flexible bronchoscope, with the help of a needle attached to catheter.[1,2] Considering the clinical picture, radiology and anatomical landmarks, mental image of the site where needle puncture is to be made is created before performing C-TBNA.[1,2]

In the Indian subcontinent, by the time patients present to the hospital, they have advanced disease with bulky lymph nodes.[1] Enobronchial ultrasound-guided TBNA (EBUS-TBNA) may not be feasible because of non-availability or cost constraints. Safety concerns and management of complications are of paramount importance with invasive procedures because of limited resources in developing countries. C-TBNA is a useful method for accessing these sites, as it is minimally invasive, safe and cost-effective. Though underutilized, it has a diagnostic role in mediastinal lymphadenopathy or peribronchial, endobronchial or peripheral submucosally located lesions.

We present the encouraging results gathered by a retrospective review of the patients subjected to C-TBNA from the year 2006 to 2016, thus reinforcing its use as a diagnostic modality.

2. Materials and methods

A retrospective review of all the patients subjected to C-TBNA's performed during the period of December 2006 to February 2016 by a single bronchoscopist in a tertiary care centre in India was done.

Patients found to be suitable for C-TBNA after analysis of the clinical picture and radiology were subjected to the procedure as a part of diagnostic work up. Discussion with the radiologist as and when required was done.

C-TBNA was done in the following patients:

- Patients who had a lymph node in the right hilar, right paratracheal or subcarinal region, the size of which was greater than 15 mm on radiology.
- Patients in whom a previous fibre-optic bronchoscopy (FOB) for brushing/biopsy of the endobronchial lesion had caused excess bleed before adequate sampling could be done or where the endobronchial lesion was feared to cause excess bleed, thus raising safety concerns.
- Patients who had an endobronchial lesion covered with necrotic slough where biopsy was either inconclusive or was expected to be inconclusive and the samples needed to be taken from deeper areas.
- Patients with submucosal lesions causing an apparent bulge when viewed through FOB, though the overlying mucosa was apparently normal looking.

These were the patients where C-TBNA was the exclusive modality utilized for diagnosis. This was done in view of the limited supply and high cost of the TBNA needle.

After obtaining the informed consent, FOB was done under local anaesthesia with fibre-optic bronchoscope with a 3.2 mm working channel. Vitals were monitored during the procedure.

C-TBNA was done with 21-gauge needle. When the needle sheath was seen, the needle was made to exit and puncture was done using either one or a combination of the established methods.[3] Three to five passes were taken routinely. Direct smears were made and fixed immediately in 95% alcohol. Few air-dried smears were also made. Samples were submitted to the pathologist for assessment. Bronchoscope was checked after every C-TBNA procedure for any damage by ''leak test''. No damage was encountered during the study period.

Data gathered by the retrospective review was critically analyzed with respect to age, sex, site of sampling and the diagnosis so achieved.

2.1. *Statistical analysis*

Discrete categorical data was represented in the form of either a number or a percentage. The normality of quantitative data was checked by measures of Kolmogorov–Smirnov tests of Normality. Mean ages of different groups were compared using One-Way ANOVA followed by Post Hoc Multiple Comparisons test. Proportions were compared using Chi-square or Fisher's exact test, depending on their applicability. Analysis was conducted using IBM SPSS STATISTICS (version 22.0). p value of <0.05 was considered to indicate statistical significance.

3. Results

161 patients underwent C-TBNA procedure from December 2006 to February 2016. In only one patient, C-TBNA failed because the patient did not cooperate, while 160 patients underwent a successful procedure. There were 111/160 (69.4%) males and 49/160 (30.6%) females.

Minor bleeding was noted in 7 patients, which was controlled by local instillation of standard commercially available lignocaine and adrenaline solution and/or cold saline and local pressure by the bronchoscope itself. No patient encountered any major complication or a need to terminate the procedure before sampling could be done.

Year-wise data of the number of C-TBNA's performed along with the patients in whom a positive yield was attained are shown in Fig. 1.

There was a drop in the number of patients who underwent C-TBNA from the later half of the year 2010 to the first half of 2013, which was due to non-availability of TBNA needles through the hospital supply.

Out of the 160 patients who underwent a successful C-TBNA, the sampling site was divided into the following 3 main categories: intraluminal bulge visible in the airways with intact mucosa, visible endobronchial growth and enlarged lymph nodes; the results are tabulated in Table 1.

Analysis of C-TBNA's from lymph nodes revealed that subcarinal node alone was targeted in 83/102 (81.4%) patients, followed by right paratracheal in 13/102 (12.7%) patients. Both the subcarinal and right paratracheal nodes together were aspirated in 5/102 (4.9%) patients. Right hilar lymph node was targeted in only 1 patient.

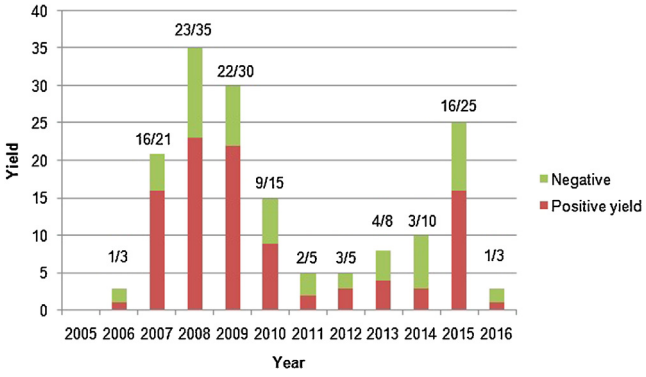

Fig. 1 – Year-wise data of the number of TBNA's performed and the diagnostic positive yield.

Procedure of C-TBNA alone revealed diagnosis in 100/160 (62.5%) patients. In 60/160 (37.5%) patients, diagnosis could not be attained either because of inadequate sampling material or a negative result. Infection was diagnosed in 30 patients, sarcoidosis in 13 patients and tumour in 57 patients (Table 2). There were no statistically significant differences between males and females as far as diagnosis was concerned ($p = 0.393$).

Amongst the infection group, tuberculosis (TB) was diagnosed in 25/30 (83.3%) patients, while amongst the tumour group, non-small cell lung cancer (NSCLC) predominated, with 37/57 (64.9%) patients (Table 2).

When NSCLC was further divided into major pathological subtypes, squamous cell carcinoma predominated with 25/37 (67.6%) diagnosed with it, followed by adenocarcinoma and non-small cell carcinoma (poorly differentiated) in 4/37 (10.8%) patients each, adenoid cystic carcinoma in 3/37 (8.1%) patients and carcinoid in 1/37 (2.7%) patient.

When site of puncture was analyzed with respect to results, it was seen that out of the 17 patients who underwent C-TBNA for bulge, 13/17 had positive yield (76.5%), out of 41 patients who underwent C-TBNA for growth, 35/41 (85.4%) had positive yield, and out of 102 patients who underwent C-TBNA for lymph node, 52/102 (51%) had a positive yield (Table 3).

Out of 17 patients who underwent C-TBNA for bulge, 10/17 (58.8%) patients had tumour as the diagnosis, followed by infection. In patients with an intraluminal growth, 33/41 (80.5%) patients were diagnosed with tumour, followed by infection. Out of 102 patients whose lymph nodes were sampled, infection was diagnosed in 25/102 (24.5%) patients, followed by tumour and sarcoidosis (Table 3).

Thus, it was found that patients with intraluminal growth or bulge had higher chances of being diagnosed with tumour and the results were found to be statistically significant ($p < 0.001$).

Detailed analysis of results with respect to sampling site is shown in Table 4.

Similarly, when the results of the C-TBNA's from right hilar, right paratracheal and both right paratracheal and subcarinal lymph node together were analyzed, it was seen that sample size of the patients was small in these 3 groups; therefore, results could not be statistically interpreted. Only the sample size of subcarinal lymph node aspirations was adequate, and yield was found to be 57.8%, which was encouraging.

When the analysis of the intraluminal findings with respect to age was done, it was found that intraluminal bulge and growth were seen predominantly in older age groups while enlarged lymph nodes were predominantly seen in younger patients, and the differences were found to be statistically significant ($p = 0.018$) (Table 5).

When the analysis of the results with respect to age was done, it was found that infection was predominantly diagnosed in younger patients with mean age being 34 years, sarcoidosis predominantly in the middle aged with mean age being 41 years, and tumour predominantly in older patients with mean age being 57 years, and the differences were found to be statistically significant ($p < 0.001$) (Table 6).

Table 1 – The sampling site (target site of TBNA procedure).

Target site	Number of patients	Percentage (%)
Bulge	17	10.6
Growth	41	25.6
Lymph node	102	63.8
Total	160	100.0

4. Discussion

C-TBNA was performed after critically selecting the patients as mentioned previously. A diagnostic yield of 62.5% was attained over an approximate ten-year period. The non-availability of in-house needles was a major constraint, as non-affordability led to a drop in the number of procedures carried out from the year 2010 to 2013, stressing upon the need for continuous

Table 2 – The diagnosis achieved in patients with a positive yield.

Diagnosis	Number of patients	Sub-category	Number of patients	Percentage within the major group
Infection	30	Fungal	2	6.7
		Pyogenic	3	10
		Tuberculosis	25	83.3
Sarcoidosis	13	Sarcoidosis	13	100
Tumour	57	NHL	2	3.5
		NSCLC	37	64.9
		SCC	18	31.6

NHL: non-Hodgkin's Lymphoma, NSCLC: non-small cell lung cancer, SCC: small cell lung cancer.

Table 3 – The diagnosis achieved with C-TBNA with respect to intraluminal findings.

Intraluminal finding		Infection	Negative	Sarcoidosis	Tumour	Total
Bulge	Count	3	4	0	10	17
	% within C-TBNA	17.6%	23.5%	0%	58.8%	100%
Growth	Count	2	6	0	33	41
	% within C-TBNA	4.9%	14.6%	0%	80.5%	100.0%
Lymph node	Count	25	50	13	14	102
	% within C-TBNA	24.5%	49.0%	12.7%	13.7%	100.0%

C-TBNA: conventional transbronchial needle aspiration.

Table 4 – The results of the TBNA procedure with respect to the sampling site.

Diagnosis		Bulge	Growth	R H	R PT	R PT + SC	SC
Infection	Fungal	0	1	0	0	0	1
	Pyogenic	0	0	0	0	0	3
	Tuberculosis	3	1	0	3	1	17
Sarcoidosis		0	0	0	1	2	10
Tumour	NHL	0	0	0	0	0	2
	NSCLC	8	26	1	0	0	2
	SCC	2	7	0	0	0	9

RH: right hilar, R PT: right paratracheal, R PT + SC: right paratracheal and subcarinal both, SC: subcarinal, NHL: non-Hodgkin's Lymphoma, NSCLC: non-small cell lung cancer, SCC: small cell lung cancer.

Table 5 – The analysis of the intraluminal findings with respect to age.

	≤20 years	21–30 years	31–40 years	41–50 years	51–60 years	61–70 years	>70 years
Bulge	1	2	0	2	6	5	1
Growth	0	3	6	8	7	14	3
Lymph node	3	18	30	18	18	11	4

Table 6 – The analysis of the results with respect to age.

	≤20 years	21–30 years	31–40 years	41–50 years	51–60 years	61–70 years	>70 years
Infection	2	12	10	3	2	1	0
Sarcoidosis	0	2	4	4	3	0	0
Tumour	0	2	5	9	14	24	3
Negative	2	7	17	12	12	5	5

supply of accessories in a developing country like ours. It is difficult for poor patients turning up at a government set-up to bear the cost of accessories, and hence, the procedure may not be carried out at all. This may lead to progression of underlying disease, adding on to morbidity and mortality.

Positive yield achieved by us encourages us to keep using it as a first-line basic diagnostic tool in selected patients, over and above EBUS-TBNA, especially when it is not available. Besides, EBUS-TBNA is a more complicated procedure and increases the risk of complications like mediastinitis, pericarditis, pneumothorax and sepsis.[2,4] It is costly and requires general anaesthesia or deep sedation.[2,4] Expertise of a bronchoscopist is also of paramount importance. Also, it is less flexible because of ultrasound probe and the bronchoscope has a larger diameter.[3]

Analysing the diagnostic results, it was seen that Tuberculosis predominated the infection group, as was expected in a high-burden country like ours.[5] It also reinforced the fact that in developing countries, patients present later with an advanced disease with bulky lymph nodes or endobronchial involvements.[1]

NSCLC was the predominant malignancy amongst the tumour group. Amongst the NSCLC, squamous cell carcinoma was seen in 67.6% patients, as again was expected in the Indian subcontinent, where patients usually come with heavy smoking histories and are predisposed to squamous and small cell cancer (SCC).[6]

When the group of patients with SCC was critically analyzed, it was found that out of the total of 18 patients with the diagnosis, 9/18 (50%) had intraluminal findings (2/18 had intraluminal bulge and 7/18 had an intraluminal growth), while in the rest of the 9/18 patients, subcarinal lymph node aspiration revealed diagnosis. When a group of patients with diagnosis of squamous cell cancer was critically analyzed, it was found that 24/25 (96%) had intraluminal findings (7/25 had intraluminal bulge while 17/25 had a visible intraluminal growth); there was only 1/25 patient where diagnosis was achieved with C-TBNA from subcarinal lymph node.

Diagnostic yield was found to be encouraging in patients with an endobronchial growth where biopsy of the lesions had failed previously or was not feasible because of issues related to excessive bleeding and its subsequent management due to

limited resources. Also, C-TBNA helped clinch diagnosis in patients with endobronchial growth covered with necrotic slough where samples were taken from deeper areas (as biopsy had been/was expected to be inconclusive). Similar encouraging findings were implicated by Kupeli as well.[2]

Also in submucosal and peribronchial lesions, where an apparent bulge was seen intraluminally, C-TBNA was of tremendous help as reflected in the positive yield so obtained. In such patients, because of overlying normal epithelium, brushing and biopsy are unhelpful in making the diagnosis.

When the analysis of results with respect to age was done, it was found that infection was predominantly diagnosed in younger patients, sarcoidosis predominantly in the middle aged, and tumour predominantly in older patients, and differences were found to be statistically significant ($p < 0.001$). This is in accordance with what is expected as a diagnosis in a particular age group.

The yield of 51% in C-TBNA from lymph node samples is also encouraging. There are various studies that implicate varying results. Kupeli et al. had implicated that results of C-TBNA can exceed 35% if mediastinal lymph nodes of more than 20 mm, located in hilar, subcarinal or right paratracheal sites, are aspirated.[7] There are various other studies that implicate a yield from 14 to 100%.[1,8]

Our results do not negate the advantages associated with EBUS-TBNA, especially when it comes to sampling of lymph nodes as we got a positive yield only in 51% lymph node sampled patients. But definitely we stress upon its use in endobronchial growths and intraluminal bulges, where biopsies may not be practically feasible, and EBUS-TBNA may be far from reality because of non-availability or cost constraints. And this is definitely in addition to its use for sampling of lymph nodes. If we review the available literature comparing EBUS-TBNA with C-TBNA, there are variable results. Some studies implicate that EBUS-TBNA has a better diagnostic yield while others find the results to be comparable to each other.[3]

When small lymph nodes less than 10 mm in their short axis are to be sampled, or the station 4L (Left lower paratracheal) nodes need to be targeted, EBUS-TBNA has a proven better yield.[9] But definitely in patients with bulky lymph nodes, endobronchial lesions and intraluminal submucosal bulges, there is a good diagnostic role of C-TBNA as seen in our results.

The expertise for the procedure is gained over time with the help of study material, workshops and hands-on-courses and it does not require any specialized formal training.[2] Proper selection of patients for the procedure in view of the clinical and radiological picture can yield good results and can thus be encouraging for the bronchoscopist as well. Also, C-TBNA is a basic diagnostic technique and helps in learning curve for a successful EBUS-TBNA procedure.

5. Conclusion

Encouraging yield achieved with C-TBNA, with minimal complications, stresses upon its use as a basic diagnostic tool. It can give confirmatory results in difficult cases with intraluminal growths and submucosal lesions. It should not be considered a blind procedure as the clinical picture, radiological imaging, intraluminal findings and anatomical landmarks guide the bronchoscopist to the appropriate site of sampling.

In a developing country like India, where EBUS-TBNA is not available in all the centres, C-TBNA should definitely be used as a basic diagnostic tool in properly selected patients. FOB with diagnostic intent is incomplete if C-TBNA is available but not done in a patient supposed to benefit from the same. Cost factor is of paramount importance in the developing world, as seen here in our analysis, where non-availability of TBNA needles led to a drop in the number of procedures. C-TBNA needle costs only around two-three thousand rupees while EBUS-TBNA needle costs over ten thousand rupees. Most of our patients were unable to afford even the conventional needle when the hospital ran out of supply. We, as a clinician, should not always aim at high-end costly procedures and equipments, but use the very basic diagnostic tools available as far as possible. Therefore, EBUS-TBNA cannot replace C-TBNA as a standard examination protocol for diagnosis of mediastinal or hilar lymphadenopathy, and this fact should always be kept in mind.[3]

Conflicts of interest

The authors have none to declare.

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Original Article

Treatment outcome of extrapulmonary tuberculosis under Revised National Tuberculosis Control Programme

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ABSTRACT

Background: Extrapulmonary tuberculosis (EPTB) constitutes 15–20% of tuberculosis cases in India. Earlier studies have evaluated treatment outcomes of EPTB with little information on outcomes of individual site of EPTB.

Aims: The objective was to study the outcome of Directly Observed Treatment Short course (DOTS) treatment of EPTB in different organ systems under Revised National Tuberculosis Control Programme.

Methods: Multi-centric retrospectives record review was carried out in three states in India. Data were collected from TB registers and analysed.

Results: Of the total 2219 patients studied, there were more males in age group 15–45. The commonest sites of EPTB were lymph node (34.4%) and pleural effusion (25.2%) followed by abdominal (12.8%) and central nervous system (CNS) (9.4%). Lymph node involvement was more common in females (58%) and pleural effusion in males (70%). Overall treatment completion rate was 84% in EPTB patients. Treatment completion was 86% in HIV negative EPTB patients compared to 66% in HIV positive patients. Individually, treatment completion rate observed as follows: lymph node 90.9%, genitourinary 92.6%, bone and joint 86%, pleural effusion 84.7%, abdominal 76% and CNS (tuberculoma and meningitis) 63.7%. The site of EPTB was not recorded in 173 (7.8%) patients.

Conclusion: Treatment outcome of EPTB was poor in HIV infected patients and those with CNS tuberculosis. More efforts are needed to improve the treatment completion rates in these groups of patients.

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1. Introduction

Extrapulmonary tuberculosis (EPTB) constitutes 15–20% of all tuberculosis (TB) cases and in human immunodeficiency (HIV) positive patients, whereas EPTB accounts for more than 50% of all cases of TB.[1–3] According to cohort analysis by Central TB Division, Ministry of Health & Family Welfare, its prevalence in India varies between 8.3–13.1% in different districts.[4] There is a rise in incidence of EPTB in industrialised and developing countries.[5–7] due to increasing HIV prevalence, availability of better diagnostic imaging modalities, laboratory facilities and specialised medical personnel as found in studies conducted in the western world and by tertiary care hospitals in India.[8]

Revised National Tuberculosis Control Programme (RNTCP) through DOTS treatment, aimed at achieving 85% treatment success rate among those who have been treated at the time of study which is presently increased to 90%. Since management guidelines are mainly aimed at pulmonary TB, it is difficult to measure treatment outcome in EPTB, and hence most of the patients are labelled as 'treatment completed' unlike 'cure' in pulmonary TB.

EPTB, because of its low infectivity, has been given low priority in RNTCP. While DOTS regimens are widely used in treatment of pulmonary TB, it may not be the same with EPTB. Some of the reasons could be as follows: fear of disease not responding to intermittent regimens of shorter durations, non-sensitization of the physicians treating EPTB about DOTS and difficulties in monitoring response to treatment. The objective of this study was to evaluate the outcomes of DOTS treatment in different organ systems other than the lung. Earlier studies have evaluated treatment outcomes of EPTB as a whole with little information of outcomes in individual organ systems. Most studies have compared the outcomes of EPTB with outcomes of pulmonary TB.[4,7–9]

2. Materials and methods

2.1. *Design and setting of study*

This was a retrospective record review conducted in three districts of three states. Data were collected from selected tuberculosis units (TUs) of districts located in three Indian states for the period of January 2010–December 2012.

Target population: Patients with EPTB at the selected districts enrolled for RNTCP on DOTS regimens were included in the study. Patients of EPTB along with pulmonary TB who were registered under DOTS and patients in whom treatment outcome was unknown were excluded from the study.

Ethical issues: Ethical clearance was obtained from the Institutional Ethical Committee of each participating centre.

Data extraction: Data on patient's age, sex, type of patient, category of anti-tubercular treatment, HIV status, site of disease and treatment outcome were extracted from the records. Whenever data were not available in the records they were recorded as 'unknown'.

Data variables were defined as per the RNTCP Guidelines.[10,11] EPTB under RNTCP is referred to TB of organs other than the lungs including pleura, lymph node, abdomen, genitourinary tract, meningitis, tuberculoma etc.[12]

1. Type of patient.
   i. *New*: A TB patient who has never had treatment for TB or has taken anti-TB drugs for less than one month is considered as a new case.
   ii. *Transferred in*: A TB patient who has been received for treatment in a TU, after starting treatment in another TU where he has already been registered.
   iii. *Others*: A patient who does not fit in any of the above categories.
2. Category of treatment.
   Standardised intermittent thrice a week dosing regimens using streptomycin (S), isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E) as follows: Category I: $2(HREZ)_3 + 4(HR)_3$, Category II: $2(SHREZ)_3 + 1(HREZ)_3 + 5(HRE)_3$ and Category III: $2(HRZ)_3 + 4(HR)_3$. (The number denotes the duration in months and subscript denotes the frequency of doses per week.)
3. Treatment outcome.
   1. *Treatment completed*: EPTB patient who has received full course of treatment and has not become smear positive during or at the end of treatment is declared as treatment completed.
   2. *Died*: Patient who died during the course of treatment regardless of cause.
   3. *Defaulted*: A patient after treatment initiation has interrupted treatment consecutively for two or more months.
   4. *Transferred out*: A patient who has been transferred to another TU/district/state and whose treatment outcome is not available is considered as 'Transferred Out'.
   5. *Treatment failure*: Any TB patient who is smear-positive at five months or more after initiation of the treatment and not put on MDR-TB treatment.
   6. *Switched over to MDR-TB treatment*: A patient who has been diagnosed as having MDRTB by an RNTCP accredited laboratory, prior to being declared as ''Failure'', and is placed on the RNTCP MDR-TB treatment regimen is said to have switched over to MDR TB treatment.
4. **HIV status** was recorded as positive, negative, or unknown (when data were not available).
5. **Site of EPTB**: Lymph node, pleura, bone and joint, genitourinary, CNS (TB meningitis and tuberculoma), Intestine or Others, which included sites other than the above such as eye, skin, military etc.

Data management: The data were pooled at the co-ordinating centre at the National Institute for Research In Reproductive Health, Mumbai. These were then captured and compiled in Epi Info™, version 7, and analysed for variable distribution and correlation between the variables.

3. Results

Data of 2219 EPTB patients were collected from the three selected districts. The patient characteristics are shown in Table 1. Of the 2219 cases, more than half of the patients (56%) were in the age group of 15–45 years. Overall prevalence of

Table 1 – Characteristics of patients.

Patient characteristics	Number of patients (2219/100%)
Age	
Median	35
Range (years)	2–90
Category	
I	1726 (77.8)
II	278 (12.5)
III	214 (9.6)
Not specified	1 (0.04)
Gender	
Male	1204 (54.3)
Female	1015 (45.7)
Type of patient	
New	1900 (85.6)
Transferred in	37 (1.7)
Others	275 (12.4)
Not known	7 (0.3)
HIV status	
Positive	111 (5)
Negative	825 (37.2)
Unknown	1283 (57.6)

EPTB was 54.3% in males and 45.7% in females (1.2:1). Lymph node TB was more prevalent in females (58.3%, $p < 0.001$), and pleural TB was predominant in males (70%, $p < 0.001$). Eighty six percent of patients were registered as 'new' patients who had not received or received less than one month anti-tubercular treatment previously. 78% received Category I treatment and 9% received Category III treatment. Category III treatment was abolished in April 2011 and 1475 of 2219 patients were recruited after that period. Only 744 patients were recruited before that which may partly account for a small percentage of Category III group of patients.

Out of 2219 patients, 936 (42%) underwent HIV testing, and of these 110 (11.8%) were found to be HIV positive. 66% of HIV positive patients completed treatment compared to 86% in the HIV negative group ($p < 0.001$).

Among the 2219 patients, extrapulmonary site of involvement was not recorded in 173 (7.8%) patients. After excluding cases in which site of involvement was not recorded, 2046 cases of EPTB were analysed for site wise outcome and is shown in Table 2. The most common sites of EPTB were lymph node (34.4%) and pleural effusion (25.2%); followed by intestine TB (12.8%), CNS (10.2%), bone and joints (9.4%) and genitourinary (2.6%) (Fig. 1). The less common sites were grouped under ''others'' which constituted 110 (5.4%) patients. These groups had a many uncommon sites, but out of these majority had involvement of eye (28 patients), followed by miliary (12), pericardial effusion (11), skin (10) and breast (6).

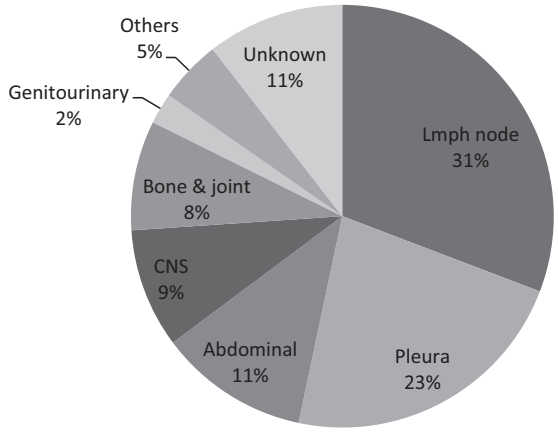

Fig. 1 – Various site involvement of extrapulmonary tuberculosis.

Overall treatment completion rate was 84%. Highest rate of treatment default (23.4%) and mortality (10%) was noted in CNS TB. Treatment failure was seen in 3 patients (0.3%) and all of them had lymph node TB (Fig. 2).

4. Discussion

EPTB, like pulmonary TB, was more prevalent (56%) in the most productive age group of 15–44 years. Lowest treatment completion (78%) was seen in the age group 55 and 64 years and this group also had maximum number of deaths (22%). Deaths in this age group can be attributed to cardio-pulmonary comorbidities or misdiagnosis with diseases like malignancy

Table 2 – Treatment outcome of extrapulmonary tuberculosis patients.

Site	Treatment outcome					
	Completed	Default	Transferred	Died	Failure	Total
Lymph node	640 (90.9)	38 (5.4)	17 (2.4)	6 (0.9)	3 (0.4)	704 (34.4)
Pleura	436 (84.7)	52 (10)	8 (1.6)	19 (3.7)	0	515 (25.2)
Abdominal	199 (76)	46 (17.5)	8 (3.0)	9 (3.5)	0	262 (12.8)
CNS	133 (63.7)	49 (23.4)	6 (2.9)	21 (10.)	0	209 (10.2)
Skeletal	165 (86)	15 (7.8)	4 (2.1)	8 (4.1)	0	192 (9.4)
Genito Urinary	50 (92.6)	1 (1.9)	3 (5.5)	0	0	54 (2.6)
Others[a]	94 (85.5)	12 (10.9)	3 (2.7)	1 (0.9)	0	110 (5.4)
Total	1717 (84.0)	213 (10.4)	49 (2.4)	64 (3.1)	3 (0.1)	2046 (100)

[a] Others include extrapulmonary sites namely, eye, miliary, pericardium, skin, breast, etc.

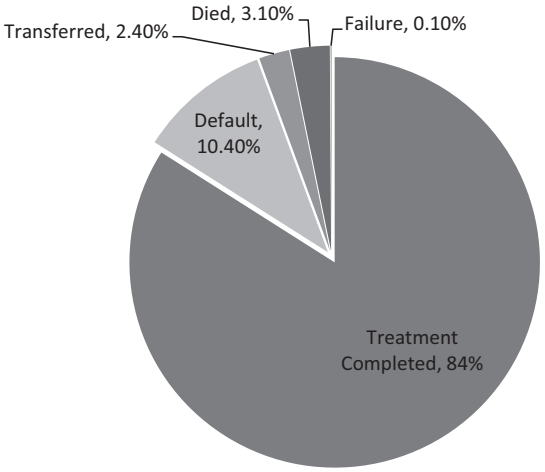

Fig. 2 – Treatment outcome of extrapulmonary tuberculosis.

which resemble TB symptomatically which are more prevalent in this age group.

Most of the existing studies done in India and abroad[7,13–16] have shown female preponderance of EPTB, and some have shown the reverse.[6,14] The present study had no significant gender differences. Females had significantly better treatment outcome than males (87% vs 82%) ($p < 0.005$). Earlier studies from India had shown that women were more likely be notified under DOTS and adhere to treatment in spite of the barriers to access of health services[17] and were less likely to default or have treatment failures.[18,19]

Lymph node TB was more common in females (58.3%, $p < 0.001$) while pleural TB was more common in males (70%, $p < 0.001$). In most of the existing studies, the commonest site of EPTB was lymph nodes, with prevalence in the range of 40–70%[4,5,7,8] followed by pleural TB. In the current study as well, lymph node TB was the commonest site (34.4%), followed by pleural TB (25.2%).

The treatment completion goal under RNTCP was 85% at the time of the study, though it has been increased to 90% in 2015 according to the National Strategic Plan of RNTCP 2012–2017.[20] Treatment completion of more than 85% was achieved in TB of lymph node (90.9%), genitourinary tract (92.6%) and skeletal (bone and joints, 86%). A higher prevalence of CNS and intestine TB was noted in this study compared to existing studies.[4,5,7,8,21] However, proportion of treatment completion was significantly lower ($p < 0.05$) in CNS (62%).and intestine TB (76%). CNS TB had the most unfavourable outcomes and highest death rate. TB of CNS causes high morbidity and mortality due to the increased chances of misdiagnosis and strong association with HIV.[22–24] Disseminated disease and the presence of CNS TB were associated with poorer prognosis as was concluded in an another study.[25]

A Finnish study had earlier concluded that the site of disease was not significantly associated with the risk of death and that other associated factors like age and comorbidities were more important risk factors for high mortality.[26]

In the current study, 85.6% of patients were registered as new patients who had not received any prior antitubercular treatment. Similar studies conducted on DOTS regimens in India had 78–88% new patients.[4,7,8] In this scenario the chances of developing MDR TB and treatment failure are low[27] and the same was demonstrated in this study. There were only three cases (0.3%) of treatment failure, and all of them were cases of TB lymph node. Treatment failure in TB lymphadenitis reported in other studies was also very low.[28,29] A study in Israel done in EPTB patients had 1.3% MDR and 5% were HIV positive.[30] Treatment failure in EPTB is infrequent compared to Pulmonary TB since EPTB is a paucibacillary disease and development of treatment failure and drug resistance is directly proportional to initial bacillary load.[27]

Among the 936 patients EPTB patients tested for HIV, 11.8% were positive. Prevalence of HIV among TB patients is around 6% in India[3] while in another large study conducted on EPTB patients, 10% were HIV infected.[5] A recent study on HIV in EPTB from Northern India showed that EPTB was the most common presentation of TB (70%) in HIV patients.[31] The prevalence of HIV positivity in EPTB patients in a study in the US was 48%.[22] HIV testing is now mandatory in every case of EPTB, and a high level of suspicion of EPTB should be maintained in an HIV infected individual. EPTB is considered to be WHO clinical stage 4 HIV disease.[12] A study undertaken in Malawi showed that patients with EPTB and HIV co-infection had low treatment completion rates.[32] This was echoed in the current study, wherein HIV negative patients showed significantly better treatment completion rates compared to HIV positive patients (86% vs. 66%, $p < 0.001$). There were no treatment failures amongst HIV positive patients but the rate of ''transferred out'' was very high in HIV positive patients (10%) compared to 2% in HIV negative. This may be because HIV positive patients may travel to a farther place for diagnosis; owing to the stigma attached to the disease and hence later be transferred out. The death rate was 12% in HIV positive patients compared to 2% in HIV negative. The HIV positive patients antiretroviral treatment (ART) status was not known. In a study among HIV-infected TB patients, treatment outcome showed low failure rates, but high case-fatality associated with lack of access to ART.[33]

Our study had some limitations. Extrapulmonary site was not available in 7.8% of patients, and results of HIV testing were not known in 60% of patients. Influence of ethnic differences between the three states was not analysed. As per the revision of DOTS regimen in April 2011, treatment categories were reduced to Category I and Category II, and Category III was stopped. This may be a confounding factor in this study, as Category III treatment was received by only 9% of the patients. In spite of these deficiencies, the results of this study can help the clinicians and programme managers in focusing on measures to improve the treatment outcome in certain group of patients with unfavourable outcomes.

5. Conclusions

The overall treatment completion was 86% in HIV negative EPTB patients using DOTS regimens. HIV positive patients showed poor compliance, and strategies to improve treatment adherence need to be explored. Unlike Pulmonary TB, guidelines for diagnosis and follow-up of patients of EPTB on treatment are not defined. Currently, the treatment guidelines

and definitions for EPTB are mere extensions of those used for Pulmonary TB. Future studies could focus on diagnostic algorithms, clinical outcomes and operational definitions for this vast and heterogeneous group. In the HIV era, EPTB should be dealt with the same enthusiasm as Pulmonary TB.

Conflicts of interest

The authors have none to declare.

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Original Article

Clinical and radiological spectrum of intracranial tuberculosis: A hospital based study in Northeast India

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ABSTRACT

Central nervous system tuberculosis (TB) is the most severe extra pulmonary TB having a high mortality and morbidity.

Objective: To study the various clinical, biochemical, and radiological spectrum of intracranial TB.

Materials and method: Ninety-three patients were enrolled in this prospective study after ethical clearance and consent from August 2013 to May 2015. The entire clinical course with complications and predictors of mortality were assessed.

Results: 36 females (38.7%) and 57 males (61.3%) were included whose mean age of presentation was 32.3 ± 17.05 years. Alcohol was the most common risk factor seen in 19.4%. Headache (90.3%) was the most common symptom. Co-infection with human immunodeficiency virus, cryptococcal, and toxoplasmosis were seen in 11, 3, and 2 patients, respectively. Cerebrospinal fluid analysis showed acid-fast bacilli in 1 patient; polymerase chain reaction for TB and BACTEC was positive in one and three patients, respectively. Neuroimaging showed basal exudates (21.7%), tuberculoma (28.6%), brain edema (27%), hydrocephalus (32.9%), infarct (21%), and abscess (2.9%). Complications were noted such as brain edema (24.7%), vasculitis (26.9%), hydrocephalus (17.2%), hyponatremia (11.8%), drug-induced hepatitis (4.3%), and drug rash in 5 patients (5.4%). A total of 25 patients (26.9%) died and 38 patients (40.9%) developed neurological sequelae like hemiparesis, paraparesis, visual loss, and hearing loss. Logistic regression showed that a Glasgow scale of <10, British Medical Research Council stage 3, and vasculitis were associated with poor outcome.

Conclusion: Lack of sensitive diagnostic method and criteria makes central nervous system TB a challenge where early diagnosis and prompt management is required.

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1. Introduction

Tuberculosis (TB) is a major public and global health issue. India is one of the world's highest TB bearing country according to World Health Organization (WHO).[1,2] It is estimated that 1% of all TB-infected patient will develop intra cranial TB in due course of time. Among the various manifestation of intracranial TB, tubercular meningitis is the most common bearing a high morbidity and mortality rate (20–60% with treatment; 100% without treatment).[3] The most important factor predicting this severity is the stage at which the patient presents and adequacy of treatment received. Definite diagnosis is made by detection of tubercle bacilli in cerebrospinal fluid, which is highly specific but lacks sensitivity,[4] because of this a multidisciplinary approach combining clinical, cerebrospinal fluid (CSF) profile and neuroimaging help us in making a diagnosis at the earliest. Uncertainty and doubts still dominates all aspect of intracranial TB. Its unpredictable natural history and various clinical manifestations pose a challenge in its diagnosis and management.

2. Materials and method

A prospective analysis of 93 patients of intracranial TB from August 2013 to September 2015 was done (informed consent and ethical clearance was obtained). The criteria are given below:

Inclusion criteria (one or more of the following):

- Patients with fever (>14 days) or any other history suggestive of TB, presence of new focal neurological deficit, or altered sensorium with past history of TB.
- Patient presenting as chronic headache with infection as a cause.
- Patient with seizure (generalized or focal) due to tuberculoma or abscess.

Exclusion criteria:

- Other causes of fever with altered sensorium or focal neurological deficit were excluded.

Detailed history, clinical examination, blood investigation, radiograph, and ultrasonography (USG) of whole abdomen was done in all the patients. Human immunodeficiency virus (HIV) and other viral markers were tested. CSF analysis like cell count, protein, sugar, adenosine level (ADA), acid-fast bacilli (AFB), gram and fungal stains, cryptococcal antigen, culture, and polymerase chain reaction (PCR) study to confirm TB and to exclude other infection was done. CSF culture was done by automated BACTEC MGIT 960 system, designed for the rapid and optimal detection of mycobacterium. The instrument photo detector measures the level of fluorescence, which corresponds to the amount of oxygen consumed by the organism. Associated test of AFB-Xpert panel was used for detection of rifampicin resistance. PCR study, which is a nucleic amplification technique, was done using MYCOREAL Real time PCR method. Neuroimaging [computed tomography (CT) head or magnetic resonance imaging (MRI) or both] was obtained in all patients.

The patients were graded into clinical stages according to British Medical Research Council (MRC)[5]:

Stage 1: includes early nonspecific symptoms and signs, without any neurological symptom.
Stage 2: Symptoms and signs of meningitis may be present, in addition to minor focal neurological deficits, isolated cranial nerve (CN) palsies, and no clouding of consciousness.
Stage 3: Patients in stupor or coma, with severe neurological deficits, seizures, posturing, and abnormal movement may be present.

Patients were treated with conventional anti-tubercular treatment (ATT) regimen, steroid, and anti-epileptics; follow up and outcome was noted. Outcome was divided into three groups: death, neurological sequelae, and complete recovery. An attempt was made to analyze various factors that would prognosticate these patients.

2.1. *Statistical methods*

Descriptive and inferential statistical analysis has been carried out. Results on continuous measurements are presented on mean ± SD (Min-Max), standard error of mean (SEM), and results on categorical measurements are presented in number (%). Significance is assessed at 5% level of significance. Chi-square/Fisher's exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

3. Results

This present study comprised of 57 males (61.3%) and 36 females (38.7%), having a male-to-female ratio of 1.6:1. The most common age group was 21–30 years; a mean age of 32.3 ± 17.05 years with a range of 2–72 years was noted. Fourteen patients (15%) were less than 14 years.

The most common risk factor was alcohol intake, seen in 18 (19.4%) patients, smoking (12.9%), diabetes mellitus (10.1%), nephritic syndrome (1.4%), systemic lupus erythematosus (SLE) (1.4%), and drug abuse and high-risk behaviors (2.2%). History of contact of TB was found in seven patients (7.5%) and a past history of TB (pulmonary or extra-pulmonary) in 23 patients (24.7%).

A total of 93 patients who were diagnosed with intracranial TB had varied presentation. The most common presentation is tubercular meningitis seen in 50 (53%) patients. Others were mixed presentation (27%), tuberculoma (15%), tubercular abscess (2.1%), and tubercular encephalopathy in one patient.

Clinical manifestations were described in Table 1. The common clinical feature noted was headache (90.3%), fever (84.9%), and meningeal sign (81.7%). The triad of meningitis (fever, headache, and signs of meningeal irritation) was found in 78.6%. Focal neurological deficit (hemiparesis, paraparesis and ataxia) and CN involvement was seen in 40 (43%) and 58 (62.4%) patients, respectively. Among the CNs, the most commonly involved is the 2nd CN (35.5%), followed by 6th (16.1%), 7th (11.8%), 3rd (7.5%), 8th (3.2%), 9th and 10th (2.2%),

Table 1 – Clinical features and examination of patients studied.

Clinical features and examination	Gender		Total ($n = 93$)
	Female ($n = 36$)	Male ($n = 57$)	
Fever	30 (83.3%)	49 (86%)	79 (84.9%)
Headache	33 (91.7%)	51 (89.5%)	84 (90.3%)
Vomiting	8 (22.2%)	10 (17.5%)	18 (19.4%)
GTCS	6 (16.7%)	12 (21.1%)	18 (19.4%)
Focal seizure	8 (22.2%)	11 (19.3%)	19 (20.4%)
Altered behavior	3 (8.3%)	10 (17.5%)	13 (40%)
Altered sensorium	20 (55.6%)	30 (52.6%)	50 (53.8%)
Focal neurological deficit	16 (44.4%)	20 (35.1%)	36 (38.7%)
Cranial nerve involvement	24 (66.7%)	34 (59.6%)	58 (62.4%)
Cerebellar signs	2 (5.6%)	2 (3.5%)	4 (4.3%)
Meningeal sign	25 (69.4%)	51 (89.5%)	76 (81.7%)
Cough	6 (16.7%)	14 (24.6%)	20 (21.5%)
Haemoptysis	1 (2.8%)	5 (8.8%)	6 (6.5%)
Abdominal distension	2 (5.6%)	1 (1.8%)	3 (3.2%)
Loss of weight	19 (52.8%)	29 (50.9%)	48 (51.6%)
Loss of appetite	20 (55.6%)	31 (54.4%)	51 (54.8%)
Lymph nodes	0 (0%)	0 (0%)	0 (0%)
Abnormal chest finding	2 (5.6%)	5 (8.8%)	7 (7.5%)
Abnormal P/A examination	2 (5.6%)	0 (0%)	2 (2.2%)

4th (1.1%), and 5th (1.1%). Six patients developed visual loss as sequelae due to secondary optic atrophy, primary optic atrophy, optico-chiasmatic arachnoiditis, occipital lobe infarct, and uveitis. Two patients develop hearing loss as sequelae. The duration of symptom ranged from 5 to 240 days.

The patients were then sub-grouped according to MRC grade; 13 patients (14%) were in MRC grade-1, and 37 (39.8%) and 43 (46.3%) were in grade-2 and grade-3, respectively. Patients with MRC grades 2 and 3 showed a bad outcome. Sixteen patients (37.2%) died and 21 (48.8%) landed up with sequelae among the 43 patients with MRC grade-3 ($P = 0.001$**, significant, Fisher's exact test, Table 2). A Glasgow coma scale (GCS) scale of <10 was seen in 15 (16.1%) patients, out of which nine patients died and two landed with sequelae. This low GCS was associated with poor outcome as in Table 3 ($P = 0.004$**, significant, Chi-square test).

CSF study was performed in all tubercular meningitis (TBM) cases. Patients with tubercular abscess and tuberculoma were not subjected to lumbar puncture. Cell count was raised in 57 patients; the mean count was 87.87 ± 159.27 cells/mm^3 with SEM of 18.64; median of 24 cells/mm^3 and a range of 2–1000 cells/mm^3. CSF cell count was normal (≤5 cells/mm^3) in 16 patients having a raised protein and low sugar. Lymphocyte predominance was noted in all except for four patients showing polymorphonuclear leucocytosis. The CSF protein was raised in most patients with a mean of 254.6 ± 312.50 mg/dl and a SEM of 36.57, median of 167, and a range of 28–2100 mg/dl. Sugar was low with a mean of 41.05 ± 25.416. CSF-ADA was elevated (≥10) in 39 patients (42%) with a mean of 15.9 ± 13, thus showing a sensitivity of 59%. AFB was positive in one patient by Ziehl–Neelsen (ZN) staining. CSF PCR study for TB was positive in one out of 35 patients performed showing a sensitivity of 2.8%. Mycobacterium culture by BACTEC was positive in three out of the 31 patients performed showing a sensitivity of 9.6%.

HIV was positive in 11 patients with a CD4 count ranging from 52 to 519. Associated co-infection with cryptococcal

Table 2 – MRC grade of patient's disease severity in relation to outcome.

MRC grade	Outcome			Total ($n = 93$)
	Death ($n = 25$)	Sequelae ($n = 38$)	Complete recovery ($n = 30$)	
Grade 1	0	4 (30.7%)	9 (69.2%)	13 (14%)
Grade 2	9 (24.3%)	13 (35.1%)	15 (40.5%)	37 (39.8%)
Grade 3	16 (37.2%)	21 (48.8%)	6 (13.9%)	43 (46.3%)

$P < 0.001$**, significant, Fisher's exact test.

Table 3 – Glasgow scale of patients during admission in relation with outcome.

Glasgow scale	Outcome			Total ($n = 93$)
	Death ($n = 25$)	Sequelae ($n = 38$)	Complete recovery ($n = 30$)	
<10	9 (60%)	2 (13.3%)	4 (26.7%)	15 (16.1%)
>10	16 (20.5%)	36 (46.2%)	26 (33.3%)	78 (83.9%)

$P = 0.004$**, significant, Chi-square test.

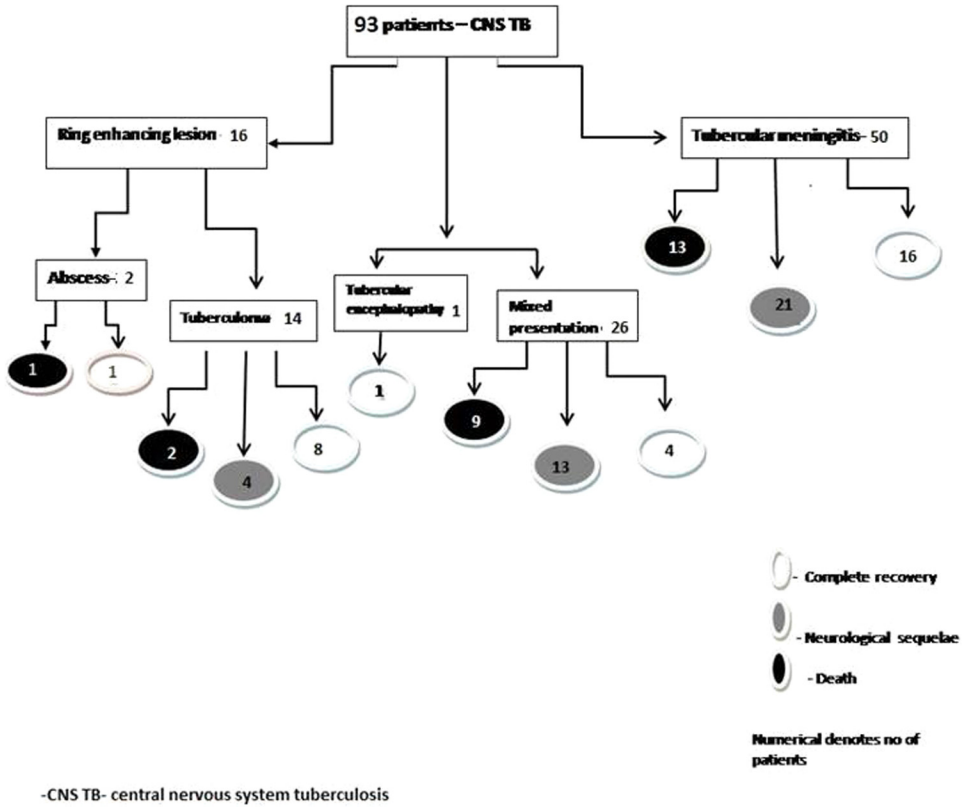

Fig. 1 – Spectrum of intracranial tuberculosis.

meningitis was seen in three patients and toxoplasmosis in two patients. Diagnosis of TBM in these patients was made on the basis of systemic supportive evidence and neuroimaging (magnetic resonance spectroscopy, MRS).

Pulmonary involvement (active and remote) was found in 31 (33.3%) patients on chest radiograph, features suggestive of abdominal Koch was seen in 13 (14%) patients on sonography with one adrenal gland involvement.

CT-scan head (plain and contrast) was done in all patients showing abnormality in 67 patients. Hydrocephalus was seen in 29 (31.2%), infarct in 12 (12.9%), ring enhancing lesion in 24 (25.8%), and basal exudates and meningeal enhancement in 11 (11.8%) patients. MRI brain (plain, contrast with MRS) was done in 47 patients. Basal exudates were seen in 24 (25.8%), infarct in 8 (8.6%), hydrocephalus in 16 (17.2%), abscess in 3 (3.2%), tuberculoma in 35 (37.6%), and brain edema in 31(33.3%) patients. Optico-chiasmatic arachnoiditis was seen in two patients (Figs. 3–6). Complications noted were brain edema (24.7%), vasculitis (26.9%), hydrocephalus (17.2%), hyponatremia (11.8%), drug-induced hepatitis (4.3%), and drug rash in five patients (5.4%). Six patients underwent ventriculo-peritoneal shunt for hydrocephalus out of which two died.

The diagnosis of central nervous system (CNS) TB was made in 24 patients solely based on neuroimaging, 57 patients based on CSF profile with AFB positive in one patient, culture positive in three patients, and a PCR positive CSF in one patient. The rest of the patients had a combination of clinical and radiological profile suggestive of TB.

A total of 25 patients (26.9%) died and 38 (40.9%) developed neurological sequelae like hemiparesis, paraparesis, visual loss, and hearing loss. The presence of hyponatremia, co-infection, development of complication like brain edema, and hydrocephalus were associated with poor outcome but statically significant results were seen with the presence of CN deficit or focal neurological deficit ($P = 0.04$), vasculitis ($P < 0.05$), low GCS ($P = 0.004$), and MRC grades 2 and 3 ($P = 0.001$).

Fig. 2 – ZN staining showing AFB (arrow) in CSF smear.

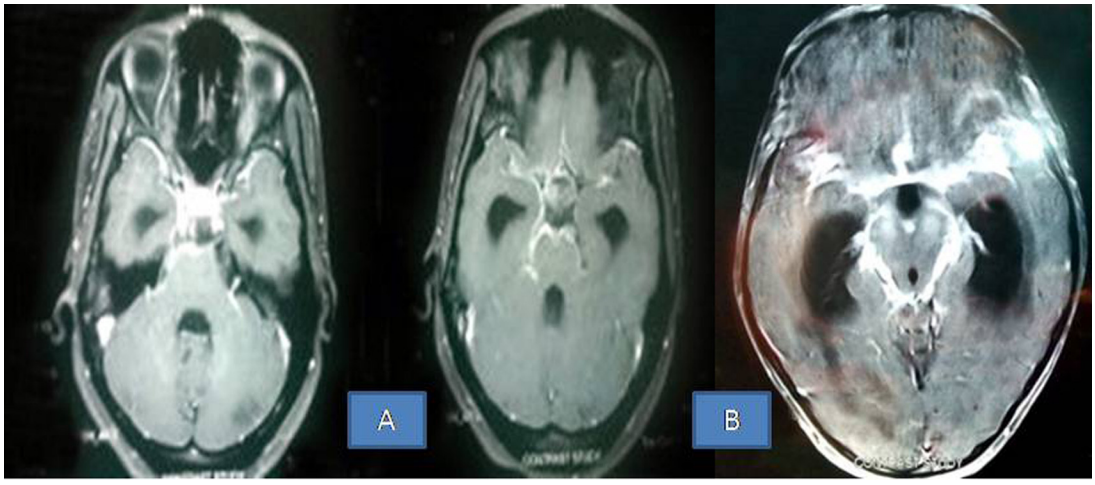

Fig. 3 – (A) Axial section of MRI brain T1 contrast study showing exudates across base of brain, optico-chiasmatic arachodinitis along with dialation of temporal horn of lateral ventricles, and fourth ventricle. (B) Axial section of MRI brain T1 contrast study showing basal exudautes encircling the brain stem ('*spider web appearance*') with hyperintesities in the mid brain area along with hydrocephalus.

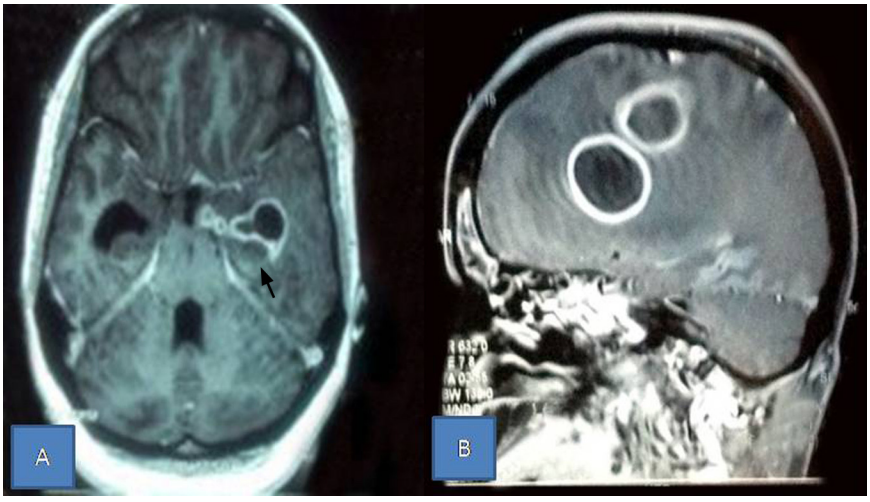

Fig. 4 – (A) Axial section MRI brain T1 contrast study showing ring enhancing lesions of left temporal lobe (arrow) with ependymal enhancement of temporal horn of left lateral ventricle with hydrocephalus. (B) Sagittal section MRI brain T1 contrast image showing ring enhancing lesions of right parietal lobe with central low signal intensity s/o abscesses (patient was HIV positive).

4. Discussion

Intracranial TB is the most severe form of extra-pulmonary involvement with high mortality and morbidity.[6] Most tuberculous infections of the CNS are caused by *Mycobacterium tuberculosis* which occur due to rupture of tuberculomas that form around *M. tuberculosis* deposited in the brain parenchyma and meninges during the initial hematogenous dissemination. CNS TB develops in two stages. Initially small tuberculous lesions (Rich's foci) develop in the CNS, later rupture or growth of one or more of these small tuberculous lesions cause the development of various types of CNS TB. Tubercular meningitis is the most common type of CNS TB among the others like tubercular encephalopathy, tubercular vasculopathy, space occupying lesions like tuberculoma, and tubercular abscess. Its incidence peaks from 0 to 4 years of age[7] and is responsible for 2–4% of all pediatric admission. In the present study, we noted only 14 patients ≤14 years of age. The most common age group noted was 21–30 with a range of 2–72 years.

TB usually does not have any sex predilection but some studies have shown a male predominance like noted in ours.[8] The most accepted hypothesis for this sex predilection is the distribution of risk factors affecting males more and a cultural diversity trait that reduces the women's ability to access health care system.

Risk factors like alcoholism, diabetes mellitus, malignancy, recent immunosuppressant drugs used to exist but HIV

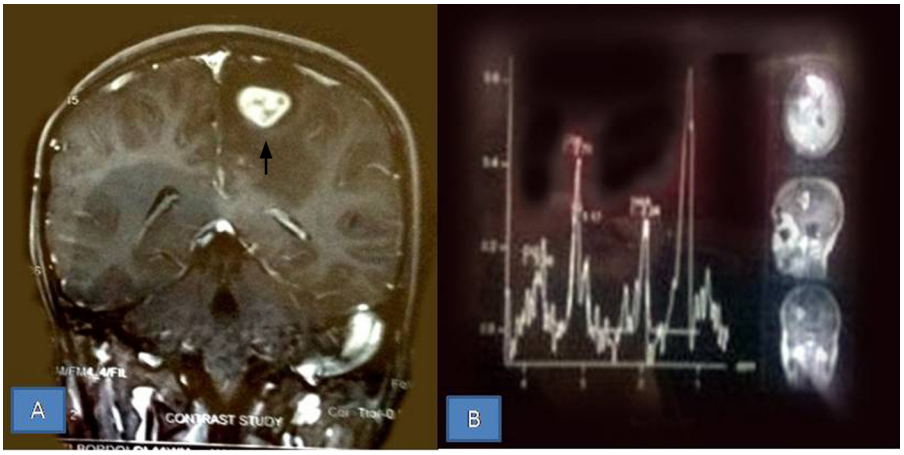

Fig. 5 – (A) Coronal section MRI brain T1 contrast study showing conglomerated ring enhancing lesions involving left parietal lobe (arrow) with perilesional edema. (B) MRS (magnetic resonance spectroscopy) of a ring enhancing lesion showing lipid and lactate peak suggestive of a tuberculoma.

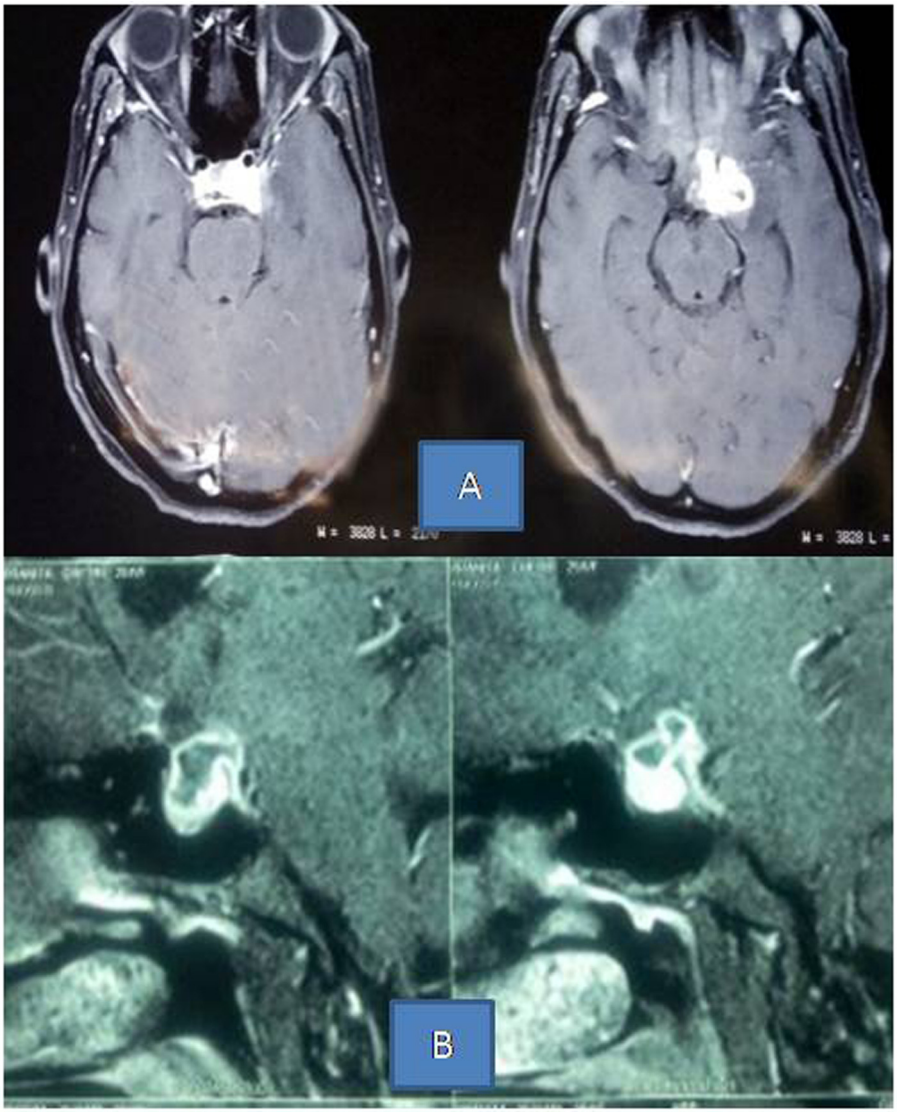

Fig. 6 – (A) Axial section MRI brain T1 contrast study showing a conglomerated heterogenous rim, nodular enhancing lesion in the left parasellar region with dense enhancing basal exudates encasing the ipsilateral internal carotid artery. (B) Sagittal section MRI brain T1 contrast study showing ring enhancing lesion involving optic chiasma with enhancement of suprasellar structure.

co-infection and appearance of multi drug resistance (MDR)-TB are the most dangerous fuels, increasing the percentage of CNS involvement up to 20%.[9]

The most common risk factor noted in the present study was alcohol intake (19.4%) but the most relevant was HIV positive status and rifampicin resistance, which was noted in 11 and one patient, respectively.

The clinical manifestations of intracranial TB are dependent on its pathogenesis. Fever and headache (60–80%) are the most common presentations; signs of meningeal irritation (adult 40–80%, children 98%) and altered sensorium are more common in children as compared to adults. However, in approximately 25–30% of cases, there may be no history of fever.

In our studies, the common clinical feature noted was headache (90.3%), fever (84.9%) and meningeal irritation sign (81.7%). The classical triad of meningitis was found in 78.6% patients. Focal neurological deficit (43%) and CN involvement (62.4%) was associated with a bad outcome ($P = 0.04$).

Seizures are reported to occur in TBM with a range of 17–93%.[10,11] Acute seizures are more common in children (50%) as compared to adults (5%).[12,13] All types of seizures may occur with etiology being multi-factorial like raised intra-cranial pressure, hydrocephalus, meningeal irritation, tuberculoma, and cerebral ischemic lesion. Sporadic cases of TBM presenting with non-convulsive status have also been reported.[14,11] In our study, seizure (focal and generalized) was noted in 37 (39.8%) patients with tuberculoma being the common etiology.

CN palsy is seen in 20–30% of TBM cases, sixth CN being the most common. If optic neuropathy were included the percentage would go up higher.[15]

Our study documented CN involvement in 58 cases (62.4%). The most common involved was 2nd (35.5%) followed by 6th (16.1%). Others were 7th, 3rd, 8th, 9th and 10th, 4th, and 5th.

The incidence of vision impairment in CNS TB varied from 27 to 72%. It may result from optico-chiasmatic arachnoiditis, compression of optic nerve or optic chiasma by tuberculoma, optic nerve granuloma, vascular optic neuropathy, anti-tubercular therapy (ethambutol, sometimes isoniazid), secondary to hydrocephalus and raised intracranial tension, bilateral occipital infarcts due to vasculitis, and local causes like chorioretinitis and uveitis.[15]

Apart from being a predictor of poor outcome,[16] presence of CN involvement also shows a positive predictive value in making the diagnosis of TBM and differentiating it from acute bacterial meningitis.[17]

Duration of symptoms before the patient seek medical help was variable with a range of 5–240 days. However, patients can also present with symptoms of less than a week.[18] Such short incubation period was explained by the Beijing genotype widely distributed in Asia. This strain was associated with increased virulence, drug resistance, increased association with HIV patients,[19–22] many outbreaks,[23] extra pulmonary involvements,[24] lesser sensitivity to Bacillus Calmette–Guérin (BCG) vaccine,[25–27] and a lower number of CSF leucocytes which in turn influence the outcome.[28]

In our study, we had varied spectrum of intracranial TB (Fig. 1). We noted 50 (53.7%) tubercular meningitis, 14 (15%) tuberculoma, 2 (2.1%) brain abscess and 1 (1.4%) with tuberculous encephalopathy; 26 (27.9%) patients had a mixed type of presentation. Of this entire group, tubercular meningitis constituted the highest mortality (13.9%) and morbidity (22.5%).

CSF examination forms an important part in the diagnosis of patients. Its abnormality will actually depend on the stage of disease and the immunity level of patients. However, CSF study may be normal initially requiring a second lumbar puncture study after 48 h.[29] A lymphocytic pleocytosis of more than 20 cells per mm^3 is usual; however, the cell count may raise up to 1000 cells or may be acellular as reported in HIV patients. In the first 10 days, polymorphic nuclear cell predominance may be found.[30]

In our series, CSF cell count was normal (≤5 cells/ml) in 16 patients and only one patient was immune-compromised. Those 16 patients with normal cell count had raised protein and low sugar. CSF-ADA was elevated (≥10) in 39 patients showing a sensitivity of 59%.

The gold standard is the detection of the bacilli by ZN smear or by isolation in culture. The sensitivity of these methods is low (20% in staining and 55% in culture) despite high specificity (98%) and is time consuming.[31–33] Nucleic-acid amplification and other PCR assays may provide a screening tool for the diagnosis of TBM. Although rapid and specific (98%), the sensitivity is low (56%) limiting its diagnostic use.[34]

In our study, AFB was positive only in one patient by ZN staining (Fig. 2). CSF PCR study for TB was positive in 1 out of 35 patients performed showing a sensitivity of 2.8%. Mycobacterium culture by BACTEC was positive in 3 out of the 31 patients performed showing a sensitivity of 9.6%.

The result of such low positivity in the present study could be because of the low amount of CSF, the day on which the sample was sent to the laboratory and technical issues. A list of factors can increase the positivity rate: at least 6 ml of CSF and direct examination of smear for at least 30 min could increase the sensitivity of the smear microscopy up to 60%.[35] Fluorescent microscopy using specified fluorochrome dye has improved the sensitivity of smear microscopy by approximately 10% and reduced the examination time.[36] On the other hand, the sensitivity of the smear examination drops from 52 to 2% after approximately 5–15 days of therapy.[37]

The Xpert MTB/RIF technology has had a significant impact on the MTB diagnosis helping us to pick up rifampicin resistant cases and also rules in test of TBM in HIV patient. It shows both high sensitivity (27–86%) and specificity (99–100%) in CSF culture.

Upcoming things like adding of Triton X 100 to identify TB bacilli by ZN stain, interferon gamma assay, microscopic observation drug susceptibility (MODS), and biomarkers like MTB specific antigen and antibody have been used for the diagnosis of TB in CSF.[38,31,39]

Neuroimaging is an important step in the evaluation of CNS TB. MRI is better than CT brain with a higher sensitivity and specificity. It can define the neuro-radiological picture of TBM and is better for evaluation of brainstem pathology, arachnoiditis around base of brain, or the optico-chiasmatic areas. The common feature noted is tuberculoma, hydrocephalus, and meningeal enhancement.[28] Presence of hydrocephalus is a poor prognostic marker associated with a higher risk of stroke.[40] Vasculitis causing ischemic infarcts is seen in 20–40% cases, mostly within basal ganglia and internal capsule regions.[41]

In our study, meningeal enhancement and basal exudates was seen in 24 (25.8%) and 11 (11.8%) on MRI-brain and CECT-brain, respectively. Hydrocephalus was seen in 36 patients on CT-scan head and MRI-brain. Among these, symptomatic hydrocephalus was seen in 16 patients with VP shunt inserted in six patients. Of these six patients, two died and two had visual loss as sequelae. Infarct was seen in 12 (12.9%) and 8 (8.6%) in CT brain and MRI brain, respectively mostly located in the capsulo-ganglionic area, fronto-parietal region, and occipital lobe. A 'tubercular zone' comprising of caudate, anterior thalamus, anterior limb, and genu of internal capsule is the most common area affected in vasculitis.[42]

Tuberculoma is a common brain parenchymal lesion. It may be solitary or multiple which is usually conglomerated. It is commonly seen in the frontal and parietal lobes, cortico-medullary junction. It may be solid caseating, solid non-caseating, liquefactive, or miliary type.[41] A ring-enhancing lesion with a hypodense center known as the 'target sign' may be considered characteristic of tuberculoma. Tuberculoma when presenting with tubercular meningitis does not create a diagnostic dilemma, but tuberculoma as a sole presentation is a major challenge. CSF examination is often normal and tissue biopsy is invasive and not feasible. Presence of a specific lipid peak on MRS and evidence of TB elsewhere in the body is the key to overcome the challange.[43]

In our study, tuberculoma was seen in 35 patients (37.6%); they were multiple located in frontal, parietal, temporal, occipital lobes, thalamus, and brainstem. Of these 35 patients, 14 patients had tuberculoma as a sole presentation. A lipid peak on MRS and evidence of TB elsewhere in the body supported the diagnosis. Tuberculoma as a sole presentation had a better outcome than those associated with meningitis ($P = 0.009$).

Tuberculous abscesses (<10%) are usually seen in the elderly and immunocompromised. They may be solitary or multiple and are frequently multiloculated. TB abscess has thinner, smoother enhancing walls, is larger (>3 cm) with peripheral edema and mass effect. We noted two tubercular abscesses of which one was an HIV patient and the other diabetic.

Intracranial co-infection with other organisms is rare and usually seen in immunocompromised state. Presence of persistent severe headache, high leukocyte count in CSF, or an immunocompromised state should alarm us.[44] Three patients in our study had cryptococcal co-infection, of which one was HIV positive and the other was a butcher. We also noted co-infection with toxoplasmosis in two patients.

The patients were treated with daily regime anti-tubercular drugs with steroids, although fluoroquinolones and amino-glycoside were added to some patients based on clinical judgment.

Complications faced in CNS TB may be disease related or treatment related. They include persistent fever (60–95%), hyponatremia (65%), acute seizure (50% in children; 5% in adults), raised intracranial pressure, development of symptomatic hydrocephalus, and vasculitis.[34]

Hyponatremia is an independent predictor of death. Cerebral salt wasting syndrome, syndrome of inappropriate antidiuretic hormone (ADH) secretion (SIADH), and adrenal insufficiency are the possible explanations.[34] Other treatment-related complications includes drug-induced hepatitis, drug rash, and drug fever.

The most common complication noted in our study was vasculitis (26.9%), which was associated with a poor outcome ($P = 0.048$*, Table 4).

The most important factor responsible for outcome in intracranial TB is the stage at which the patient presents, those with MRC stage-3 had a mortality rate of 50–70% and neurological sequelae in 20–30% of survivors. The common neurological sequelae were hemiparesis, monoplegia, seizure, mental retardation, psychiatric sequelae, ataxia, visual loss, hearing loss, and ophthalmoplegia.

In the present study, 25 patients (26.9%) died and 38 (40.9%) developed neurological sequelae like hemiparesis, paraparesis, visual loss, and hearing loss.

Increasing age, presence of CN deficit, focal neurological deficit, and altered sensorium at presentation predicted the neurological sequelae.[45]

We had 25 deaths, out of which 16 were of MRC grade-3 and nine of grade-2. Sequelae were seen in 38 patients, of which 21 were in MRC grade-3, 13 in grade-2, and four in grade-1. Complete recovery was seen in 30 patients. A GCS of <10 was seen in 15 (16.1%) patients, of which nine died and two had sequelae.

Age >40 years, altered sensorium at presentation, seizure, absence of headache, stroke, low GCS and high MRC grade at admission, delay in initiating treatment, hyponatremia, CN involvement, high CSF cell count, high CSF protein, a low

Table 4 – Complication correlation with outcome.

Complication	Outcome			Total ($n = 93$)	P value
	Death ($n = 25$)	Sequelae ($n = 38$)	Complete recovery ($n = 30$)		
Vasculitis	7 (28%)	14 (56%)	4 (16%)	25 (26.9%)	0.048[a]
Brain edema	9 (39.1%)	7 (30.4%)	7 (30.4%)	23 (24.7%)	1.000
Hydrocephalus	8 (50%)	3 (18.8%)	5 (31.3%)	16 (17.2%)	1.000
Hyponatremia	4 (36.4%)	4 (36.4%)	3 (27.3%)	11 (11.8%)	1.000
Drug hepatitis	1 (25%)	1 (25%)	2 (50%)	4 (4.3%)	0.592
Drug rash	1 (20%)	2 (40%)	2 (40%)	5 (5.4%)	0.656

[a] Fisher's exact test applied.

glucose, high lactate level in CSF, hydrocephalus, use of mechanical ventilation, presence of HIV co-infection, and detection of MDR are factors influencing the outcome of CNS TB.[46–49]

In our study, univariate analysis revealed low GCS (<10), MRC grades 2 and 3, presence of vasculitis and infarct, presence of focal neurological deficit, and CN involvement are associated with poor outcome (statistically significant).

5. Conclusion

Intracranial TB is the most severe extra pulmonary site of involvement. In the present study, we noted hurdle in the diagnosis of CNS TB due to lack of definite diagnostic test and criteria. We also noted that prompt diagnosis of the disease and recognition of its various complications with early institution of therapy is of outmost importance in intracranial TB.

Conflicts of interest

The authors have none to declare.

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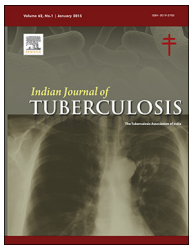

Review Article

Pyridines: Multidrug-resistant tuberculosis (MDR-TB) inhibitors

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ABSTRACT

Mycobacterium tuberculosis (MTB) infection has become an increasing health threat due to the worldwide emergence of multidrug-resistant MTB (MDR-MTB) strain. Isoniazid (pyridine) resistance problem is a complex process and is associated with mutations in several genes. However, the emergence of isoniazid (INH) resistant *M. tuberculosis* strains dictates the necessity for redesigning this old drug in order to create analogs effective against INH-resistant strains by using rational approach. In light of these findings, the present review discusses the synthesis, structural optimization, and modification in pyridine structure to combat the problem of multidrug-resistant tuberculosis.

1. Introduction

Tuberculosis (TB) is still a challenging worldwide health problem; the pathogen responsible for TB uses diverse strategies to survive in a variety of host lesions and to evade immune surveillance.[1] A key question is how robust are our approaches to discovering new novel TB drugs, and what measures could be taken to reduce the long and protracted clinical development of new drugs. There is an urgent need for the treatment of *Mycobacterium tuberculosis* more efficiently and to overcome MDR (multidrug resistance) and XDR (extensive drug resistance).[2–4]

The World Health Organization (WHO) reported that globally 3.5% of naive infections already expressed resistance to the two most efficacious frontline agents used to treat the disease, *i.e.* RIF (rifampicin) and INH (isoniazid), thereby classifying the infection as multidrug-resistant tuberculosis (MDR-TB); multidrug-resistant tuberculosis (MDR-TB) is defined as a form of TB infection caused by bacteria that are resistant to treatment with at least two of the most powerful first-line anti-TB drugs, isoniazid (INH) and rifampicin (RMP).[5–10] Treatment of drug-susceptible Mtb is difficult already, requiring 6–9 months of combination therapy. Treatment for MDR-TB can extend upwards of 2 years and relies on more toxic, less efficacious second- or third-line agents, many of which are even scarcer than frontline drugs in affected areas.[11,12] A very common and deadly complication of Mtb infection is coinfection with human immunodeficiency virus (HIV).[13] This is particularly troublesome because RIF, a mainstay in Mtb therapy, is a potent inducer of drug-metabolizing enzymes, including cytochrome P450 (CYP)3A4. This induction dramatically reduces plasma levels of several highly active antiretroviral therapy drugs; thus, patients are often forced to complete their TB treatment before beginning HIV treatment.[14–18] Patients who contract MDR-TB with HIV

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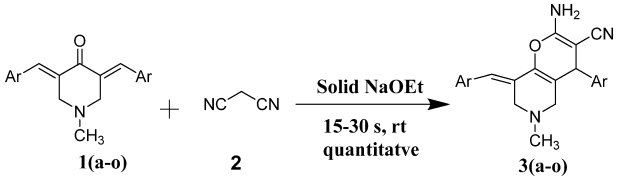
have a very poor prognosis due to the duration of treatment; these individuals frequently succumb within a few months.

Pyridine is important nucleus in isoniazid and nicotine structure; the pyridine nucleus is an important heteroaromatic class of compounds with wide range of activities. The potential antimicrobial properties of pyridine have been previously demonstrated; isoniazid (INH) remains a key component in all multiple drug treatment regimens recommended by the WHO albeit resistant isolates are rapidly generated during monotherapy or inappropriate treatment.[19–22] Hence, improvement of INH by introducing chemical modifications in its core structure in order to enhance the biological response against Mtb and/or circumvent resistance phenomena continues to be an interesting scientific challenge.[23–26]

In the current literature, we are reporting synthesis of pyridine analog against multidrug-resistant tuberculosis along with their SAR and effective potent pyridine derivatives against *M. tuberculosis*, with improved properties such as enhanced activity against MDR strains, reduced toxicity, and furthermore, with these compounds not showing any signs of cytotoxicity.

2. WHO 2015 report on MDR-TB[27]

Globally, an estimated 3.3% of new TB cases and 20% of previously treated cases have MDR-TB; a level that has changed little in recent years. In 2014, an estimated 190,000 people died of MDR-TB. More TB patients were tested for drug resistance in 2014 than ever before. Worldwide, 58% of previously treated patients and 12% of new cases were tested, which was up from 17% to 8.5% respectively in 2013. This improvement is partly due to the adoption of rapid molecular tests. If all of the TB cases notified in 2014 had been tested for drug resistance, an estimated 300,000 would have been found to have MDR-TB, with more than half of them (54%) occurring in India, China, and the Russian Federation. The number of cases detected (123,000) worldwide represented just 41% of this global estimate, and only 26% of the 480,000 incident cases of MDR-TB estimated to have occurred in 2014. Detection gaps were worst in the Western Pacific Region, where the number of cases detected was only 19% of the number of notified cases estimated to have MDR-TB (the figure for China was 11%). A total of 111,000 people started MDR-TB treatment in 2014, an increase of 14% compared with 2013. The ratio of patients enrolled in treatment to patients newly notified as having MDR-TB or rifampicin-resistant TB was 90% globally. The ratio was above 90% in 15 of the 27 high MDR-TB burden countries as well as in the European Region and the Region of the Americas. Globally, only 50% of MDR-TB patients were successfully treated. However, the 2015 treatment success target of ≥75% for MDR-TB patients was reached by 43 of the 127 countries and territories that reported outcomes for the 2012 cohort, including three high MDR-TB burden countries (Estonia, Ethiopia, and Myanmar). Extensively drug-resistant TB (XDR-TB) had been reported by 105 countries by 2015. An estimated 9.7% of people with MDR-TB have XDR-TB.



Scheme 1 – Synthesis of 4-*H* pyrans *3(a–o)*.

3. MDR-TB inhibitors

Kumar et al. synthesized series of tetrahydro-4*H*-pyrano[3,2-c] pyridine derivatives **3(a–o)** by the reaction of a series of 1-methyl-3,5-bis[(*E*)-arylmethylidene]tetrahydro-4(1*H*)-pyridinones **1(a–o)** and malononitrile **(2)** in the presence of solid sodium ethoxide at ambient temperature under solvent-free conditions (Scheme 1). This reaction affords solely tetrahydro-4*H*-pyrano[3,2-c]pyridines **3(a–o)** without any side product, and hence neither crystallization nor column chromatographic purification is necessary.[28]

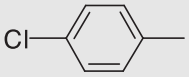
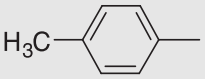
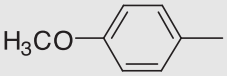
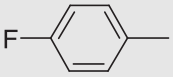
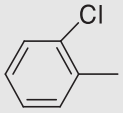
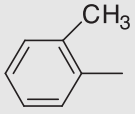
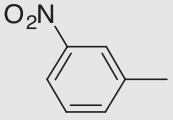
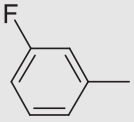
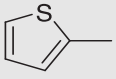
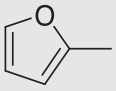
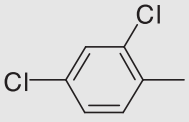
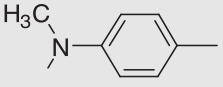
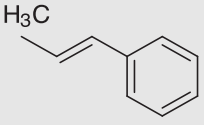
The compounds were tested for their *in vitro* activity against *Mycobacterium tuberculosis* H37Rv (MTB) and multidrug-resistant tuberculosis (MDR-TB). Compound **(3n)** 2-amino-4-[4-(dimethylamino)phenyl]-8-(*E*)-[4(dimethyl-amino)phenyl] methylidene-6-methyl 5,6,7,8 tetrahydro-4*H*-pyrano[3,2-c]-pyridine-3-carbo-nitrile was found to be the most potent compound (MIC: 0.43 μM) against MTB and MDR-TB, being 100 times more active than standard isoniazid against MDR-TB among the synthesized compounds.[28]

Structure–activity relationship demonstrated that the antimycobacterial activity is enhanced by the presence of weakly electron-withdrawing groups like chloro and fluoro in the aromatic rings (**3b**, **3e**, **3f**, and **3j**), while the presence of two chlorines at second and fourth positions (**3m**) diminished the activity. Strongly electron-withdrawing group, nitro at the third position of the aromatic ring, is uninfluential leading to the retention of good activity (**3i**). Electron-donating groups at the aryl rings reduce the activity greatly, except in (**3n**). Replacement of phenyl by other heterocyclic rings also reduces the activity markedly (**3k–l**) (Table 1).[28]

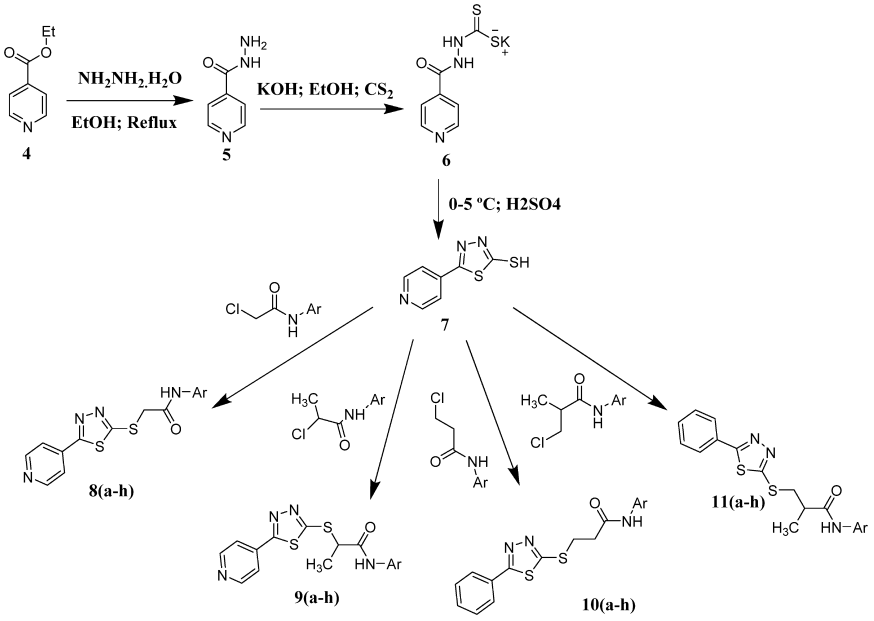
Mahajan et al. synthesized a series of pyridinyl-thiadiazoles **8(a–h)**, **9(a–h)**, **10(a–h)**, and **11(a–h)** as reported in Scheme 2. In brief, the ester **(4)** to hydrazide chemotransformation was carried out by using hydrazine hydrate in ethanol at reflux condition. The hydrazide **(5)** was then transformed to thiosemicarbazate **(6)** by usual CS_2, KOH, and ethanol method. The resultant solid was then cyclized using proton donar (sulphuric acid) at temperatures ranging from 0 to 5 °C. The pyridinyl-thiadiazole ''parent'' **(7)** then reacted with various chloro-substituted compounds for further analog synthesis. These reactions led us to the thio-substituted array of compounds **8(a–h)**, **9(a–h)**, **10(a–h)**, and **11(a–h)**. Synthesized series revealed a potent compound **(10f)**, which was comparable with isoniazid against H37Rv and MDR-TB (Tables 2 and 3).

SAR studies revealed that an increase in the length of alkyl chain with amide linkage intact (**8a**) has shown improved potency. Chloro substitution on phenyl (Compound **8e** and **8f**) results in improved activity. Similar attempt with the benzyl substitution (**8c** and **8d**) was disappointing with no further increase in activity. Assuming chlorine has vital role to play in

Table 1 – Antimycobacterial activity of 3(a–o).

Compound	R	Yield (%)	MIC (μM)		
			MTB	MDR TB	MC[2]
3a		99	35.21	NT	70.42
3b		98	0.92	1.84	3.68
3c		99	16.32	NT	65.27
3d		99	30.12	NT	60.24
3e		99	0.97	0.97	31.97
3f		98	1.84	3.68	58.96
3g		99	16.32	NT	32.64
3i		98	1.75	1.75	56.18
3j		98	0.99	1.99	31.97
3k		99	68.12	NT	34.06
3l		99	37.31	NT	37.31
3m		98	25.63	NT	50.71
3n		99	0.43	0.43	28.34
3o		99	30.71	NT	30.71
Isoniazid	–	–	0.36	45.57	45.57
Gatifloxacin	–	–	2.08	8.34	2.08

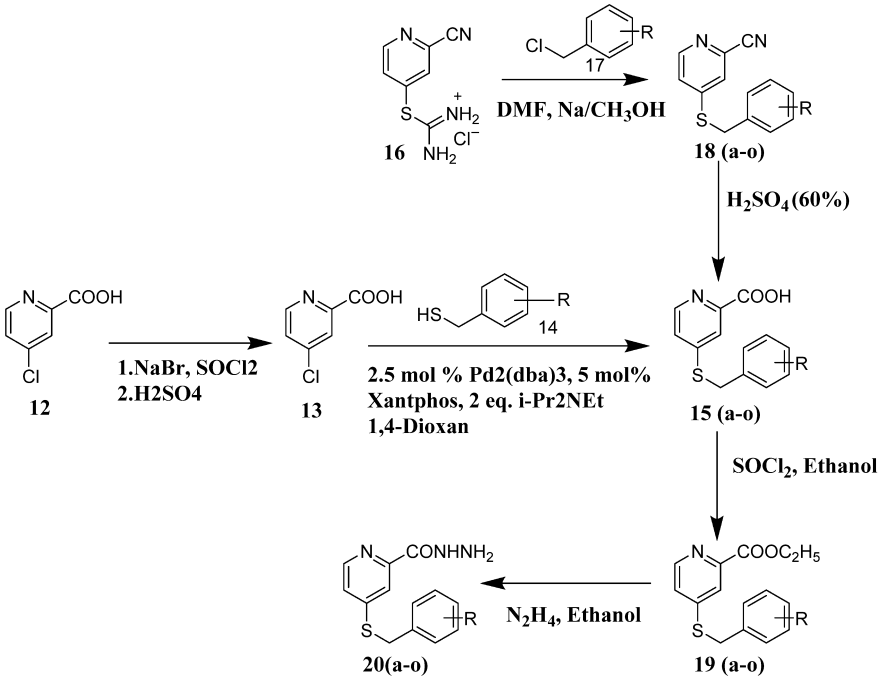
MC[2] = *Mycobacterium smegmatis* ATCC 14468.



Scheme 2 – Synthesis of pyridinyl-thiadiazoles *8(a–h)*, 9(a–h), 10(a–h) and 11(a–h).

the orientation and receptor bindings, **8g** and **8h** were synthesized but failed to reflect the potentiation.[25]

Herzigov et al. synthesized series of 4-benzylsulfanylpyridine-2-carbohydrazides, following the steps depicted in Scheme 3. Since it was not possible to prepare the desired sulfides **(20)** directly either by the reaction of 4-mercaptopyridine-2-carbohydrazides with benzyl halides or 4-chloropyridine-2-carbohydrazides with benzylthiols, they synthesized sulfides **(20)** *via* the key intermediates **(15)**.[15] These 4-benzylsulfanylpyridine-2-carboxylic acids **(15)** were formed *via* two routes. The first method used for the preparation of **(15)** was based on Pd-catalyzed cross-coupling of 4-chloropyridine-2-carboxylic acid **(13)** with benzylthiols **(14)**. Acid **(13)**, which serves as a convenient starting material, was prepared from pyridine-2-carboxylic acid **(12)**. Thiols **14** were prepared by heating appropriate benzyl halides with thiourea in ethanol,



Compound 15, 18, 19 and 20 Whre a = H; b = 3-Cl; c = 4-Cl; d = 3-F; e = 4-F; f = 3-Br; g = 4-Br; h = 3-CH3; i = 4-CH3 ; j = 3-CF3; k = 4-CF3; l = 3-CN; m = 3-OCH3; n = 4-NO2; o = 3,5-(NO2)2

Scheme 3 – Synthesis of the 4-benzylsulphanyl derivatives.

Table 2 – Antimicrobial activity of 8(*a–h*), 9(a–h), 10(a–h), and 11(a-h).

Compound	Ar	MIC_{50} (μM)	MBC_{90} (μM)	CC_{50} (μM)
8a	$-C_6H_5$	0.18 ± 0.08	1.25–2.5	15.21 ± 2.65
8b	$-CH_2-C_6H_5$	0.19 ± 0.07	1.25–2.5	17.65 ± 0.68
8c	$-CH_2$–2-ClC_6H_4	9.67 ± 1.45	ND	ND
8d	$-CH_2$–4-ClC_6H_4	11.72 ± 3.57	ND	>50
8e	–2-ClC_6H_5	8.52 ± 1.33	ND	>50
8f	–4-ClC_6H_4	6.81 ± 1.08	ND	>50
8g	–2,5-$Cl_2C_6H_3$	>20	ND	ND
8h	–2,4-$Cl_2C_6H_3$	>20	ND	ND
9a	$-C_6H_5$	1.48 ± 1.28	5–10	>50
9b	$-CH_2-C_6H_5$	1.15 ± 1.03	5–10	>50
9c	$-CH_2$–2-ClC_6H_4	1.26 ± 1.19	5–10	>50
9d	$-CH_2$–4-ClC_6H_4	>20	ND	ND
9e	–2-ClC_6H_5	1.04 ± 0.01	5–10	>50
9f	–4-ClC_6H_4	0.12 ± 0.46	1.25–2.5	>50
9g	–2,5-$Cl_2C_6H_3$	1.19 ± 0.39	5–10	>50
9h	–2,4-$Cl_2C_6H_3$	2.45 ± 1.22	5–10	>50
10a	$-C_6H_5$	0.61 ± 0.13	2.5–5	>50
10b	$-CH_2-C_6H_5$	0.67 ± 0.24	2.5–5	13.97 ± 0.71
10c	$-CH_2$–2-ClC_6H_4	0.65 ± 0.15	2.5–5	>50
10d	$-CH_2$–4-ClC_6H_4	0.63 ± 0.24	2.5–5	15.62 ± 0.81
10e	–2-ClC_6H_5	0.62 ± 0.17	2.5–5	>50
10f	–4-ClC_6H_4	0.07 ± 0.04	1.25–2.5	7.26 ± 0.51
10g	–2,5-$Cl_2C_6H_3$	0.59 ± 0.11	2.5–5	12.6 ± 0.84
10h	–2,4-$Cl_2C_6H_3$	0.55 ± 0.18	2.5–5	>50
11a	$-C_6H_5$	2.26 ± 0.84	5–10	>50
11b	$-CH_2-C_6H_5$	2.51 ± 1.36	5–10	>50
11c	$-CH_2$–2-ClC_6H_4	1.89 ± 0.86	5–10	>50
11d	$-CH_2$–4-ClC_6H_4	1.68 ± 0.68	ND	ND
11e	2-ClC_6H_5	2.05 ± 1.06	5–10	>50
11f	4-ClC_6H_5	0.22 ± 0.5	1.25–2.5	>50
11g	2,5-$Cl_2C_6H_3$	1.55 ± 0.64	5–10	>50
11h	2,4-$Cl_2C_6H_3$	1.48 ± 0.66	5–10	>50
Isoniazid		0.03 ± 0.01	ND	ND

The inhibitory activity (MIC50) was determined against *M. tuberculosis* H37Rv. The cidal activity (MBC90) and cytotoxicity (CC50) were determined after 5 days of exposure to a single dose of compound. Assays were carried out at least two times. MIC50: Minimum Inhibitory Concentration 50%; MBC90: Minimum Bactericidal Concentration 90%, CC50: cyototoxic concentration 50%. n.d.: not determined.

Table 3 – MDR – TB activity of compound 10*f*.

Strain	Drug resistance	**10f** MIC (μg mL^{-1})
TN565	R,S,EM,K,Cl	0.06
TN576	I,R,S,EM,ET,K	0.06
TN702	I,S,EM,P	0.25
TN715	I,S,EM,P	0.06
TN768	I,R,S,EM,ET	0.06
TN772	I,R,S,EM	0.03
TN1195	I,R,S,EM	0.25
TN1314	I,R	0.03
TN1618	I,R,S,EM,ET,CI	0.06
TN1811	I,R,S,EM	0.03
TN2557	I,R,S,EM,ET,CA	0.13

The **10f** susceptibilities were also tested on 9 multidrug resistant (MDR) and 2 polyresistant MTB strain. (b) Twenty of the twenty-five sensitive and resistant clinical isolates tested were previously determined to be genetically distinct by IS6110 genotyping. I, isoniazid; R, rifampin; S, streptomycin; EM, ethambutol; ET, ethionamide; K, kanamycin; P, pyrazinamide; Cl, ciprofloxacin; CA, capreomycin.

and the resulting *S*-alkylisothiouronium salts were further hydrolyzed by an aqueous solution of sodium hydroxide. The treatment of **(13)** with various benzylthiols **(14)** was carried out in dioxane and iPr2NEt in the presence of catalyst $Pd_2(dba)_3$. The process required 9–12 h depending on the alkylating agent and furnished products **(15)** in 32–68% yields.

This procedure failed for the derivatives with the nitro group on the benzyl moiety. For this reason, they used the formerly prepared sulfide-nitrile, which was converted to the key intermediates **(15)** by hydrolysis in 60% sulfuric acid in good yields. Sulfides **(18)** were obtained by reacting isothiouronium salt **(16)** with the appropriate benzyl halides **(17)** in *N*,*N*-dimethylformamide in the presence of sodium methoxide, *i.e.*, the second method for the formation of a C-S bond. The reaction of **15** with $SOCl_2$ in ethanol led to the formation of esters **(19)**, which gave the target hydrazides **(20)** by the reaction with hydrazine.

The series of 4-benzylsulfanylpyridine-2-carbohydrazides was synthesized and evaluated for *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis*, nontuberculous

Table 4 – *In vitro* antimycobacterial activity of 4-(benzylsulfanyl)pyridine-2-carbo hydrazide expressed as MIC (μmol/L) against MDR-TB.

Compound	MDR-TB strains					
	M. tuberculosis 7357/98[a]		*M. tuberculosis* 9449/06[b]		*M. tuberculosis* 2092/05[c]	
	14d	**21d**	**14d**	**21d**	**14d**	**21d**
20c	4	8	4	8	2	4
20g	4	4	4	4	4	8
20m	4	8	8	16	8	16
20n	8	8	8	8	4	8
20o	>16	>62	>31	>62	32	>125

[a] Resistant to isoniazid, rifampicin, streptomycin, ethambutol, ofloxacin, and ansamycin.
[b] Resistant to isoniazid, rifampicin, streptomycin, ethambutol, and ansamycin.
[c] Resistant to isoniazid, rifampicin, streptomycin, ethambutol, ofloxacin, and ansamycin.

Table 5 – MDR and XDR-Mtb activity of compounds 29, 33, 38 and control PA-824.

Strain[a]	Compound MIC μM (μg/ml)			
	29	33	38	PA-824
DS-Mtb1	0.04–0.07	0.8 (0.3)	<0.03 (<0.01)	0.5–0.9
DS-Mtb2	0.04–(<0.01)	0.8 (0.3)	<0.03 (<0.01)	>13.9 (>5)
MDR-Mtb HREZSKP	0.04–(<0.01)	6.5 (2.5)	0.03–0.8[b]	0.5–0.9
MDR-Mtb HREKP	0.04–(<0.01)	0.4 (0.2)	<0.03 (<0.01)	0.5–0.9
MDR-Mtb HRERb	2.3 (0.63)	13–26 (5–10)	0.80 (0.31)	0.5–0.9
XDR-Mtb HRESKO	0.04–(<0.01)	0.8 (0.3)	<0.03 (<0.01)	0.9 (0.3)
XDR-Mtb HREKO	0.04–(<0.01)	0.8 (0.3)	<0.03 (<0.01)	0.2 (0.08)

[a] DS-Mtb1 = drug-sensitive clinical isolate no. 1; DS Mtb2 = drug-sensitive clinical isolate no .2; MDR-Mtb = multidrug-resistant Mtb; XDR-Mtb = extensively drug-resistant Mtb; drugs abbreviated as H = isoniazid, R = rifampin, E = ethambutol, Z = pyrazinamide, S = streptomycin, K = kanamycin, P = p-aminosalicylic acid, Rb = rifabutin, O = ofloxacin.
[b] Growth inhibited > 90% up to 0.8 μM (0.3 μg/mL). MICs are the minimum concentration required to inhibit growth by > 99%; MICs were done in 7H9/glucose/glycerol/BSA/0.05% Tween 80 and the average of three individual measurements.

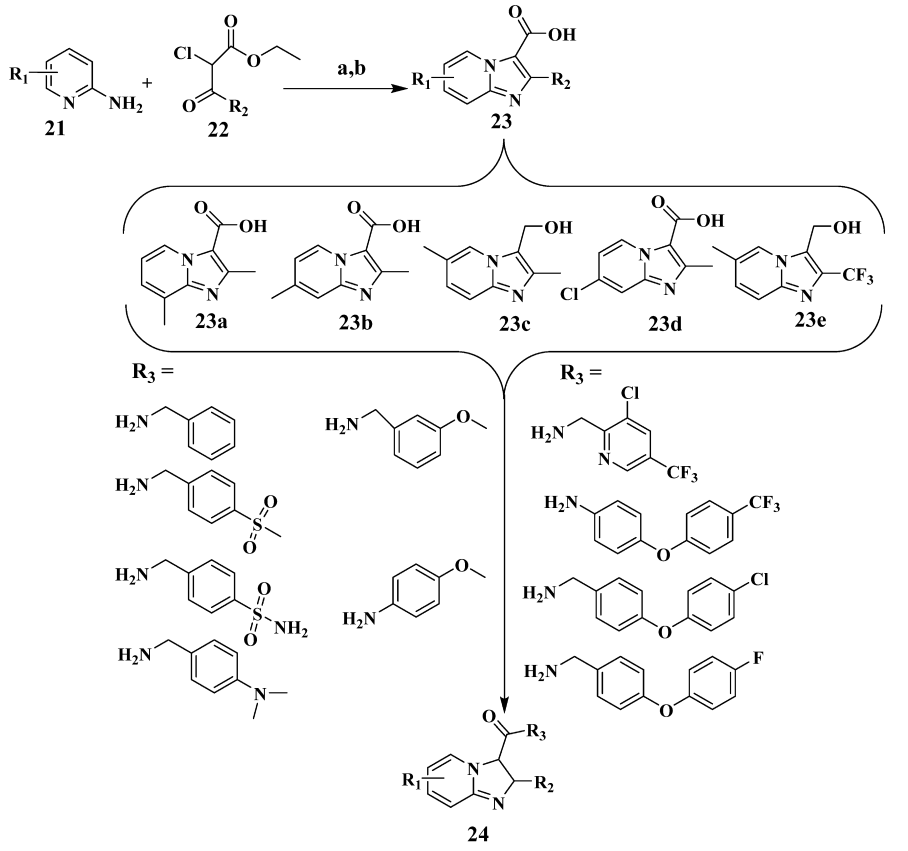
mycobacteria, and multidrug-resistant *M. Tuberculosis* (MDR-TB). Compound **20c**, **20g**, **20m**, **20n**, **20o** showed the activity against the different strains of *Mycobacterium tuberculosis*, i.e.7357/98, 9449/06, 2092/05. Minimum inhibitory concentration (MIC) falls into a range of 2–125 μmol/L, most often 4–32 μmol/L (Table 4). 4-Benzylsulfanylpyridine-2-carbohydrazide derivatives having electron-withdrawing substituents cause the increase of the activity. Especially incorporating two nitro groups into the benzyl moiety led to the most active compounds **(20n)**. However, in our newly prepared series, compound **(20o)** having two nitro groups on the benzyl moiety displays poor activity. The exact values of MICs could not be determined due to the insolubility of compound **(20o)** in the test medium. They assume that the low efficacy of this compound is connected with its low solubility.[29]

Moraski et al. synthesized a series of imidazo[1,2-a] pyridine-3-carboxamides derivatives by simple and straightforward strategy to develop the designed molecules, outlined in Scheme 4. They prepared five different imidazo[1,2-*a*] pyridine-3-carboxylic acid intermediates **23(a–e)**. First, reaction of the appropriately substituted 2-aminopyridine **(21)** with ethyl 2-chloroacetoacetate followed by saponification with lithium hydroxide and acidic work-up gave the free acids **23(a–d)**. Use of the same procedure but starting with 2-amino-5 methyl pyridine **(21)** and ethyl 2-chloro-4,4,4-trifluoroacetoacetate [**(22)**, R_2=CF_3] followed by saponification and acidic work-up gave free acid **(23e)** (87% yield). It should be noted that while carboxylic acids **23(a–e)** can all be prepared by the general method described, they also are commercially available, while **(23e)** was not. Finally, these imidazo[1,2-a] pyridine-3-carboxylic acid intermediates were all readily converted to various amide analogs **(25–39)** through classical EDC-mediated coupling reactions.[30]

All the synthesized compounds were screened for *in vitro* against *Mycobacterium tuberculosis* H37Rv; among them, compounds **29**, **33**, and **38** were found to be potent against a panel of MDR and XDR drug-resistant clinical Mtb strains with the potency of **(38)** surpassing that of clinical candidate PA-824 by nearly 10-fold. These results indicate that readily synthesized imidazo [1,2-*a*]pyridine-3-carboxamides are an exciting new class of potent, selective anti-TB agents.

The SAR focus was to make compounds with lower cLog P values than compound **(25)** (cLog P of 3.6) in the hope that the more polar compounds would be substantially more potent through favorable interactions and also be more soluble. Therefore, sulfonamide **(26)**, sulfonyl **(27** and **28)**, and 2-pyridyl **(33)** compounds were made and screened. Compound **(29)** is a positional isomer of compound **(25)**. Neither compound **(29)** nor **(33)** lowered the cLog P, but they did add chemical diversity to this series (Table 5).[30]

Navarrete-Vazquez et al. synthesized series of 4-(5-substituted-1,3,4-oxadiazol-2-yl)pyridine derivatives **40–55** (Scheme 5). Compounds **(40)** and **(41)** were prepared using acetic and trifluoroacetic acid, respectively, and INH. A catalytic amount of

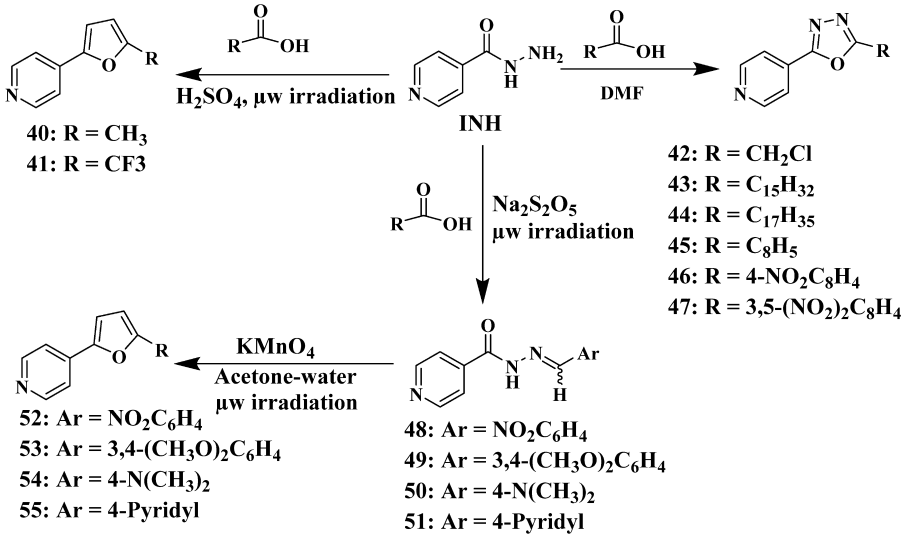


25,R_1 = 7-CH_3, R_2 = CH_3, R_3 = benzylamino
26,R_1 = 7-CH_3, R_2 = CH_3, R_3 = (4-Sulfamoylbenzyl) amino
27,R_1 = 6-CH_3, R_2 = CF_3, R_3 = (4-methylsulfonylbenzyl)amino
38,R_1 = 6-CH_3, R_2 = CH_3, R_3 = (4-methylsulfonylbenzyl)amino
29,R_1 = 6-CH_3, R_2 = CH_3, R_3 = benzylamino
30,R_1 = 7-CH_3, R_2 = CH_3, R_3 = (3-mithyloxybenzyl)anilino
31,R_1 = 7-CH_3, R_2 = CH_3, R_3 = (4-(4-trifluoromethyl)phenoxy)anilino
32,R_1 = 6-CH_3, R_2 = CH_3, R_3 = (4-(dimethylamino)benzyl)amino
33,R_1 = 7-CH_3, R_2 = CH_3, R_3 = (3-Chloro-5-(trifluoromethyl)pyridine-2-yl(methyl)amino
34, R_1 = 7-Cl, R_2 = CH_3, R_3= (3-methyloxybenzyl)amino
35,R_1 = 7-Cl, R_2 = CH_3, R_3 = (4-(4-trifluorophenoxy)benzyl)amino
36,R_1 = 7-CH_3, R_2 = CH_3, R_3 = (4-(4-trifluorophenoxy)benzyl)amino
37,R_1 = 6-CH_3, R_2 = CH_3, R_3 = (4-(4-Chlorophenoxy)benzyl)amino
38,R_1 = 7-CH_3, R_2 = CH_3, R_3 = (4-(4-fluorophenoxy)benzyl)amino
39, R_1 = 8-CH_3, R_2 = CH_3, R_3 = (4-(4-fluorophenoxy)benzyl)amino

Scheme 4 – (a) Ethyl 2-chloroacetoacetate for *23(a–d)* and ethyl 2-chloro-4,4,4-triuoroacetoacetate (for 23e), DMF, reflux, 48 h; (b) (1) LiOH, EtOH, (2) HCl, 56 h; (c) EDC, DMAP, R_3, CH_3CN, 16 h.

sulfuric acid was added to promote the dehydration and intramolecular cyclocondensation *via* microwave irradiation with low yields (<35%). Compounds **(42–47)** were obtained by treatment of INH with acyl chlorides in DMF, through one-pot *N*-acylation and cyclodehydration. Reaction of INH and different aldehydes in presence of sodium metabisulfite and dimethoxyethane afforded *N*1-(arylmethylene) isonicotinohydrazides **(48–51)**, which were used immediately in a subsequent step without purification. Oxidation of *N*1-(arylmethylene) isonicotino-hydrazides **(48–51)**, with potassium permanganate in a mixture of acetone and water (5:1) under microwave irradiation yielded compounds **(52–55)**.[31]

The synthesized compounds were evaluated for their *in vitro* antimycobacterial activity. Compound **(43)** [4-(5-pentadecyl-1,3,4-oxadiazol-2-yl)pyridine] was 10 times are active than isoniazid, 20 times more active than streptomycin, and 28 times more potent than ethambutol against drug-resistant strain CIB 112. Some compounds showed an interesting activity against *Mycobacterium tuberculosis* H37Rv and five clinical isolates (drug-sensitive and -resistant strains). Structure–activity relationship studies reveal that compound **(46)**, substituted with a 4-nitro phenyl moiety, did not show significant activity against any *M. tuberculosis* strain (MIC = 29.85 μM), whereas its regioisomeric compound **(43)** (2-nitrophenyl-substituted) showed MICs ranging from 3.76 to 7.46 μM (Table 6). The presence of additional nitro group in compound **(47)** (3,5-dinitrophenyl-substituted) resulted in a 2-fold less potency against CIBIN 687 strain, but a 2-fold improvement in activity against CIBIN112 strain compared to compound **(52)**.



Scheme 5 – Synthetic pathway of 4-(5-substituted-1,3,4-oxadizol-2-yl)pyridines (40–55).

Compound **44** showed 10 and 20 times more potency than INH strain. These compounds bear a highly lipophilic chain bonded to the fifth position of oxadiazole moiety.

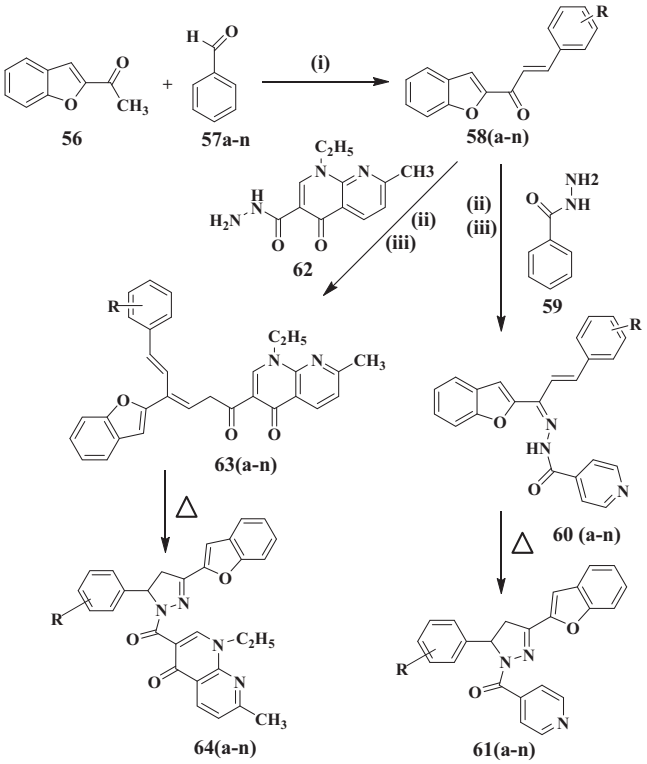
Kuntal et al. synthesized series of 3-benzofuran-5-aryl-1-pyrazolyl-pyridylmethanone and 3-benzofuran-5-aryl-1-pyrazolylcarbonyl-4-oxo-naphthyridin analogs **61(a–n)** and **64(a–n)** by microwave irradiation method.[18,32]

First crucial intermediates 1-benzo[*b*]furan-2-yl-3-phenyl-2-propen-1-ones (chalcone) **58(a–n)** were synthesized from 2-acetyl benzofuran **(56)** with various aromatic aldehydes **57(a–n)**. The chalcones were further treated with two different hydrazides, isonicotinic acid hydrazide **(59)** and nalidixic acid hydrazide **(62)** to give corresponding final compounds 3-benzofuran-5-aryl-1-pyrazolyl pyridylmethanones **61(a–n)** and 3-benzofuran-5-aryl-1-pyrazolylcarbonyl-4-oxo-naphthyridins **64(a–n)**, respectively (Scheme 6). Synthesized compounds were further evaluated for *in vitro* antitubercular activity against multidrug-resistant *M. tuberculosis* strains and found that compound **(64j)** NO_2 substituted 3-benzofuran-5-aryl-1-pyrazolylcarbonyl-4-oxo-naphthyridin was the most potent antitubercular agent against *M. tuberculosis* (MIC value = 3.6 µg/mL), even better than standard drug isoniazid and comparable with rifampin. The structure–activity relationship (structure-MTB/MDR activity) study demonstrated compounds **61(a–n)** and **64(a–n)** exhibiting good to moderate antitubercular activity. The naphthyridin ring is the more favorable group then

Table 6 – Physicochemical data and *in vitro* antimycobacterial activity of 4-(5-substituted-1,3,4-oxadizol-2-yl)pyridine derivatives against *M. tuberculosis* $H_{37}Rv$ and two drug-sensitive and three drug-resistant clinical isolates.

Compound	R	$H_{37}Rv$	*M. tuberculosis* clinical isolate MIC (µM)				
			CIBIN 687	CIBIN 650	CIBIN 675	CIBIN 234	CIBIN 112
40	$-CH_3$	49.69	6.21	49.69	49.69	49.69	49.69
41	$-CF_3$	37.21	37.21	37.21	37.21	37.21	37.21
42	$-CH_2Cl$	41.03	41.03	41.03	41.03	41.03	41.03
43	$-C_{15}H_{31}$	0.35	0.70	0.09	11.19	22.38	2.80
44	$-C_{17}H_{35}$	0.65	0.65	0.16	10.37	20.75	2.59
45	$-C_6H_5$	8.97	4.48	8.97	35.87	35.87	35.87
46	4-$NO_2C_6H_4$	29.85	29.85	29.85	29.85	29.85	29.85
47	2-$NO_2C_6H_4$	7.46	3.73	7.46	29.	29.85	29.85
52	3,5-$(NO_2)_2C_6H_3$	25.54	6.39	25.54	12.77	25.54	12.77
53	3,4,5-$(NO_2)_2$ OC_6H_3	14.12	3.53	7.06	14.12	28.25	14.12
54	4-$N(CH_3)_2$ C_6H_3	3.76	3.76	30.04	30.04	30.04	30.04
55	4-Pyridyl	8.93	4.46	8.93	35.71	35.71	35.71
Isoniazid	–	0.44	0.91	0.91	29.19	58.38	29.19
Streptomycin	–	0.86	nd	0.10	6.87	55.02	55.02
Rifampicin	–	0.07	nd	nd	0.94	121.51	3.79
Ethambutol	–	9.80	nd	nd	nd	nd	78.31

CIBIN 687 = sensitive to all first-line antitubercular agents; CIBIN 650 = sensitive to all first-line antitubercular agents; CIBIN 675 = sensitive to streptomycin, isoniazid; CIBIN 234 = sensitive to streptomycin, isoniazid, rifampin, and pyrazinamide; CIBIN 112 = sensitive to streptomycin, isoniazid, and ethambutol.



Scheme 6 – Synthetic pathway of compound *61(a–n)* and 64 (a–n). Reagent and condition: (i) EtOH, 10% KOH/20%NaOH sol., M.W.10–25 min (ii) CH_3COOH 6–10 h. (iii) CH_3COOH M. W. 12–22 min.

pyridinylcarbonyl ring for the potent activity. Carboxylic group containing compound **(61d)** was found more active against multidrug-resistant *M. tuberculosis*. Hence, the acidic medium is favorable for the formation of isonicotinoyl-NAD complex, which is produced by carboxylic group. The electron-withdrawing group (–NO_2 groups) containing naphthyridine ring compound **(64j)** produced better activity than presence of halogen, furan, and other groups in same ring system. Nitro derivatives of pyrazoline containing benzofuran with naphthyridine or pyridines are highly favorable moieties for antitubercular activity (Table 7).[32]

4. Conclusion

Tuberculosis (TB) is a communicable disease caused by *Mycobacterium tuberculosis* (Mtb). TB remains a major health risk globally, and ranks as the second cause of death from infectious disease worldwide. The current MDR-TB epidemic is the result of decades of neglect for an important infectious disease, lack of resources for national TB control programs, poor case detection, and inadequate/inappropriate therapy in high-burden countries. Initial outbreaks of MDR-TB in developed countries were associated with very high mortality rates both in HIV-negative and HIV-coinfected patients. The increasing incidence of MDR-TB and the recently reported XDR-TB pose dangerous public health problems around the world, and new pharmaceutical agents are needed to control tuberculosis. Isoniazid (pyridine) resistance problem is a complex process and is associated with mutations in several genes. So there is a pressing need for new chemotherapeutic agents to combat the emergence of drugs resistance and shorten duration of treatment to improve the patient compliances. Hence, improvement of INH by introducing chemical modifications in its scoffold structure in order to enhance the biological response against MDR-TB, resistance phenomena continues to be an interesting scientific challenge. In light of these findings, the present review discusses the synthesis, structural optimization, and modification in pyridine structure to combat the problem of multidrug-resistant tuberculosis. Based on this progress, if we can successfully leverage the opportunities in this target, there is hope that we will be able to raise MDR-TB inhibitors in earnest in the long term.

Table 7 – *In vitro* antituberculosis activity and cytotoxic result of the entire compound against MTB and MDR TB.

Compound	R	MTB[a] MIC (μg/ml)	MTB[b] MIC (μg/ml)	IC_{50} [c] (μM)
61a	–OH (o)	3.7	6.2	>195.2
61b	–OCH_3(o)	6.5	5.7	>202.4
61c	–$N(CH_3)_2$ (p)	9.2	11.5	>210.7
61d	–COOH (o)	2.2	3.2	>170.9
61e	–NO_2(m)	7.5	6.9	>240.5
61f	–OH(o), OCH_3(p)	3.3	4.5	>190.7
61g	–OH(p)	4.5	6.2	>200.3
61h	–Cl (p)	11.5	10.6	>220.7
61i	–Cl (o)	10.6	9.5	>210.6
61j	–NO_2 (o)	8.2	7.5	>245.4
61k	–OCH_3(p)	6.8	5.5	>200.3
61l	–H	8.5	9.5	>209.6
61m	Furan Ring	9.6	10.4	>208.4
61n	–CH═CH–Ar	8.6	7.5	>212.2
64a	–OH (o)	1.2	6.4	>157.6
64b	–OCH_3(o)	5.5	8.5	187.5
63c	–N(CH3)2(p)	9.2	10.6	206.8
64d	–COOH (o)	4.5	7.8	>196.4
64e	–NO_2(m)	2.3	37	>164.5
64f	–OH(o), OCH_3 (p)	4.2	5.4	>154.4
64g	–OH (p)	3.2	3.8	>148.5
64h	–Cl (P)	8.3	6.3	>160.5
64i	–Cl (o)	7.8	8.4	>178.4
64j	–NO_2 (o)	1.9	3.6	>85.4
64k	–OCH_3(p)	6.3	8.5	>156.5
64l	–H	9.8	10.8	>210.4
64m	Furan ring	7.5	8.5	>185.5
64n	–CH═CH–Ar	5.5	7.4	>168.6
Rifampicin		0.60	4.2	>74.5
Isoniazid		0.32	8.5	>130.5

[a] *M. tuberculosis* strain $H_{37}RV$.
[b] Multidrug-resistant *M. tuberculosis*.
[c] Cytotoxicity in VERO cell line.

Conflicts of interest

The authors have none to declare.

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Case Series

Linezolid-induced optic neuropathy in XDR pulmonary TB: A case series

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ABSTRACT

Optic neuropathy has been reported as a side effect of long-term use of linezolid. This is particularly seen in cases of extensively drug resistant tuberculosis (XDR-TB) where treatment with linezolid may continue for about 24–30 months. We, hereby, report two cases of XDR-TB treated patients with a regimen containing linezolid who developed progressive painless loss of vision during the course of treatment. In both the cases, the visual symptoms resolved completely on withdrawing linezolid. Early recognition of this rare side effect and timely withdrawal may salvage the eyesight of such patients.

1. Introduction

The emergence of drug-resistant tuberculosis over the past two decades has profoundly handicapped the efforts to control the global tuberculosis epidemic. The poor potency and tolerability of the second line drugs combined with the long duration of treatment further have worsened the grim scenario. Linezolid is a bacteriostatic agent of the oxazolidinone class which acts by reversible inhibition of monoamine oxidase. It is approved for use in infections due to vancomycin-resistant *Staphylococcus aureus*, *Enterococcus faecium*, nosocomial and community acquired pneumonia and various skin and skin structure infections.[1] It is also a Group 5 antitubercular agent. Neuropathy, as a side effect of linezolid, has been reported with prolonged therapy. We report two cases of linezolid-induced optic neuropathy who were on treatment for extensively drug resistant tuberculosis (XDR-TB).

2. Case 1

A 35-year-old male patient, non-diabetic, non-smoker, non-alcoholic, with XDR pulmonary TB presented with painless progressive loss of vision for 1 week. He was receiving treatment with capreomycin (750 mg/day), amoxycillin clavulinic acid (2 g/day), moxifloxacin (400 mg/day), linezolid (600 mg/day), Para-aminosalicylate (PAS) (12 g/day), terizidone

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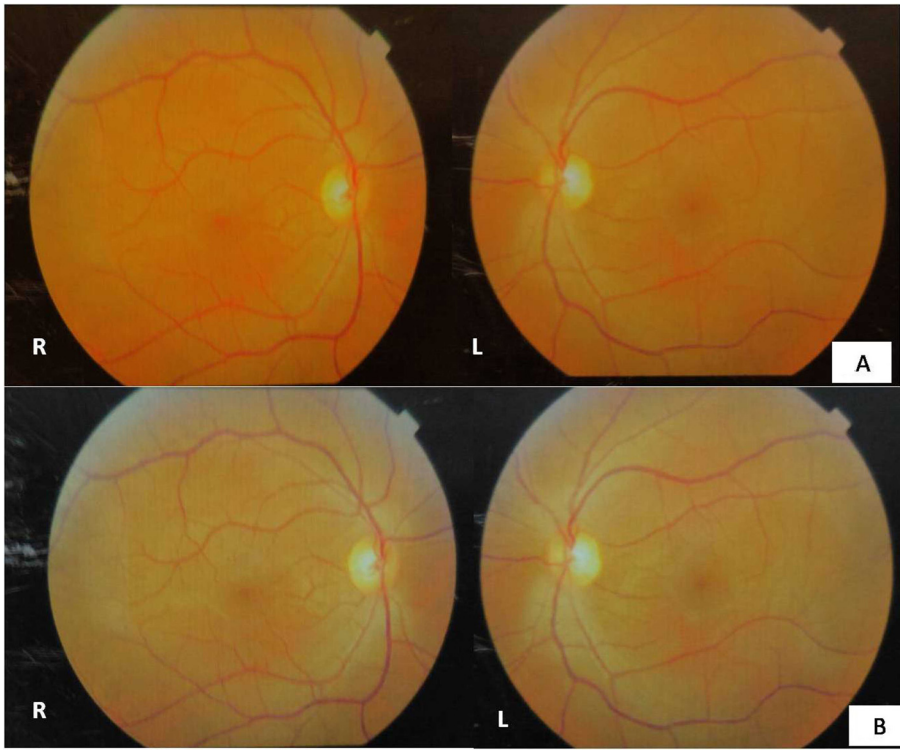

Fig. 1 – Abnormal appearance of fundus during the phase of active retrobulbar neuritis (A) with post-retrobulbar neuritis optic atrophy (B).

(500 mg/day), clofazamine (200 mg/day) and isoniazid (900 mg/day), given as per weight, for the past 10 months. He was not on any other drug with known ophthalmic toxicity.

On examination, his visual acuity was 6/24 in the right eye and 6/36 in the left eye. Colour vision was defective in both eyes. Anterior segment examination was within normal limits. Fundus examination showed features of active retrobulbar neuritis (Fig. 1A). Because of poor vision, it was not possible to capture an optical coherence tomography (OCT) in the diseased state.

Occurrence of toxic optic neuropathy due to intake of linezolid was suspected, and the drug was immediately withdrawn. The patient improved symptomatically after 1 week of stopping linezolid and free of all visual symptoms by the end of 2 weeks. Visual acuity was 6/6 in both eyes, and colour vision was restored. Fundus examination showed post-retrobulbar neuritis optic atrophy (Fig. 1B). OCT was done to look for residual nerve damage. It showed retinal nerve fibre layer thinning in both eyes (Fig. 3). Other than the minor nerve damage, regular follow-up of the patient for 1 year showed no deficit in vision.

3. Case 2

A 26-year-old male patient, non-smoker, non-diabetic, non-alcoholic, with XDR pulmonary TB presented with complaints of diminution of vision for 10 days on his monthly follow-up visit. He was on treatment with a regimen comprising imipenem (1 g/day) (as culture Drug Sensitivity Test (DST) showed capreomycin resistance), isoniazid (700 mg/day), pyrazinamide (1250 mg/day), terizidone (750 mg/day), PAS (12 g/day), clofazamine (200 mg/day) and linezolid (600 mg/day); all were given as per weight, and he had completed 5 months of therapy. We did not give capreomycin to this patient as the culture DST showed resistance to capreomycin. Owing to constraints in procuring thioacetazone, we kept the patient on imipenem. There was no history of any other drug intake.

On examination, visual acuity was reduced to finger counting at 1 m and colour vision was defective. Fundus examination showed tilted optic disc with mild features of retrobulbar neuritis (Fig. 2A). A Roth's spot inferior to optic disc was found incidentally in left eye. On stopping linezolid, patient's visual symptoms improved within 1 week. Visual acuity returned to 6/6 in both eyes. On examination of the fundus, mild temporal disc pallor was seen which was suggestive of post-retrobulbar neuritis optic atrophy. The Roth's spot had cleared (Fig. 2B). OCT with macular thickness cube revealed retinal nerve fibre layer thinning in both eyes (Fig. 4).

Patient is on regular follow-up for tuberculosis and has no visual complaints any further.

4. Discussion

Toxic optic neuropathy is defined as a clinical syndrome characterised by papillomacular bundle damage, central or cecocentral scotoma, and reduced colour vision.[1] The condition is usually easy to identify but often missed or recognised at a stage when restoration of normal vision is not possible.

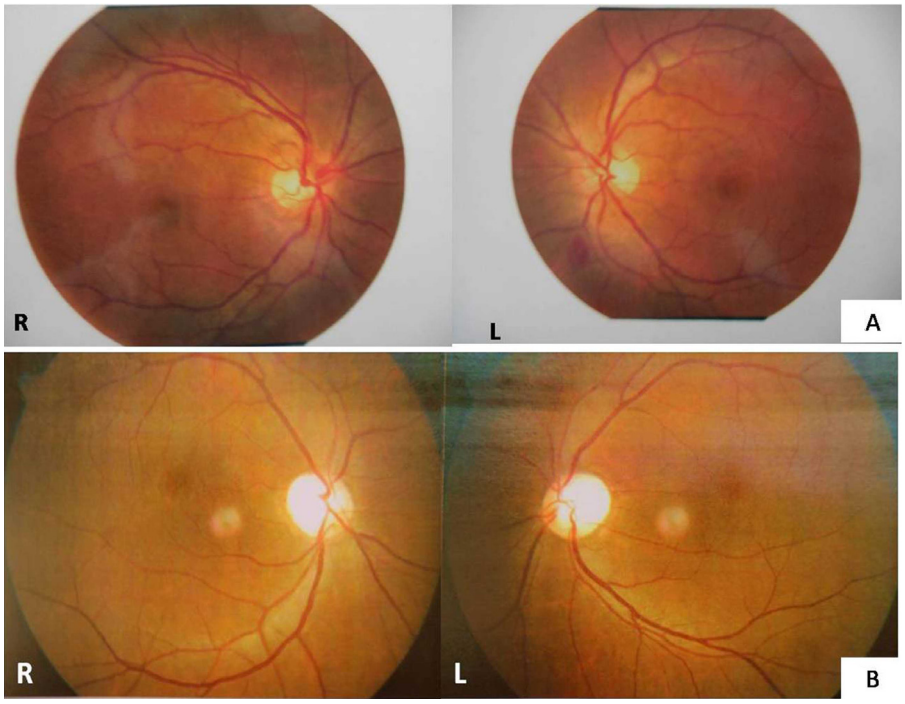

Fig. 2 – Abnormal appearance of fundus during phase of active retrobulbar neuritis (A) with post-retrobulbar neuritis optic atrophy (B). Incidental finding of Roth's spot in LE – (A) has disappeared in fig.

Common causes of toxic optic neuropathy include alcohols such as methanol and ethylene glycol; antibiotics such as chloramphenicol; antimalarial, anticancer agents; heavy metals such as lead and mercury; besides carbon monoxide and tobacco.[2] It has also been reported in vitamin B12 and thiamine deficiency.

Linezolid binds to the 23S subunit of the 50S ribosome and inhibits protein synthesis. The exact mechanism of neural

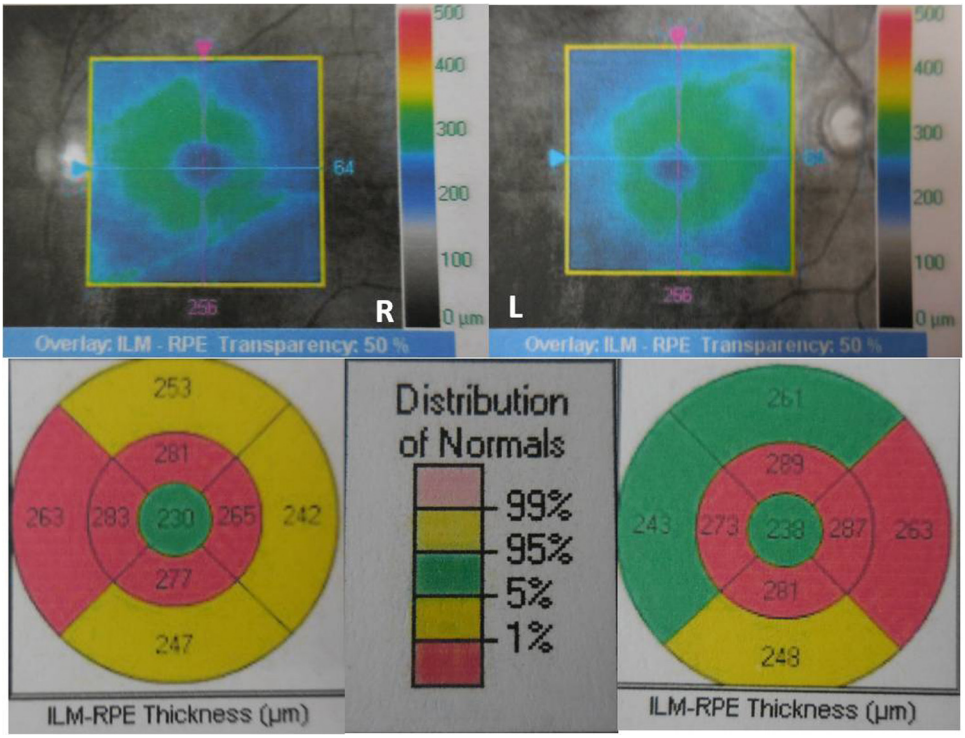

Fig. 3 – Optical coherence tomography (OCT) analysis of the macular area. Macular thickness cube showing retinal nerve fibre layer thinning (red areas).

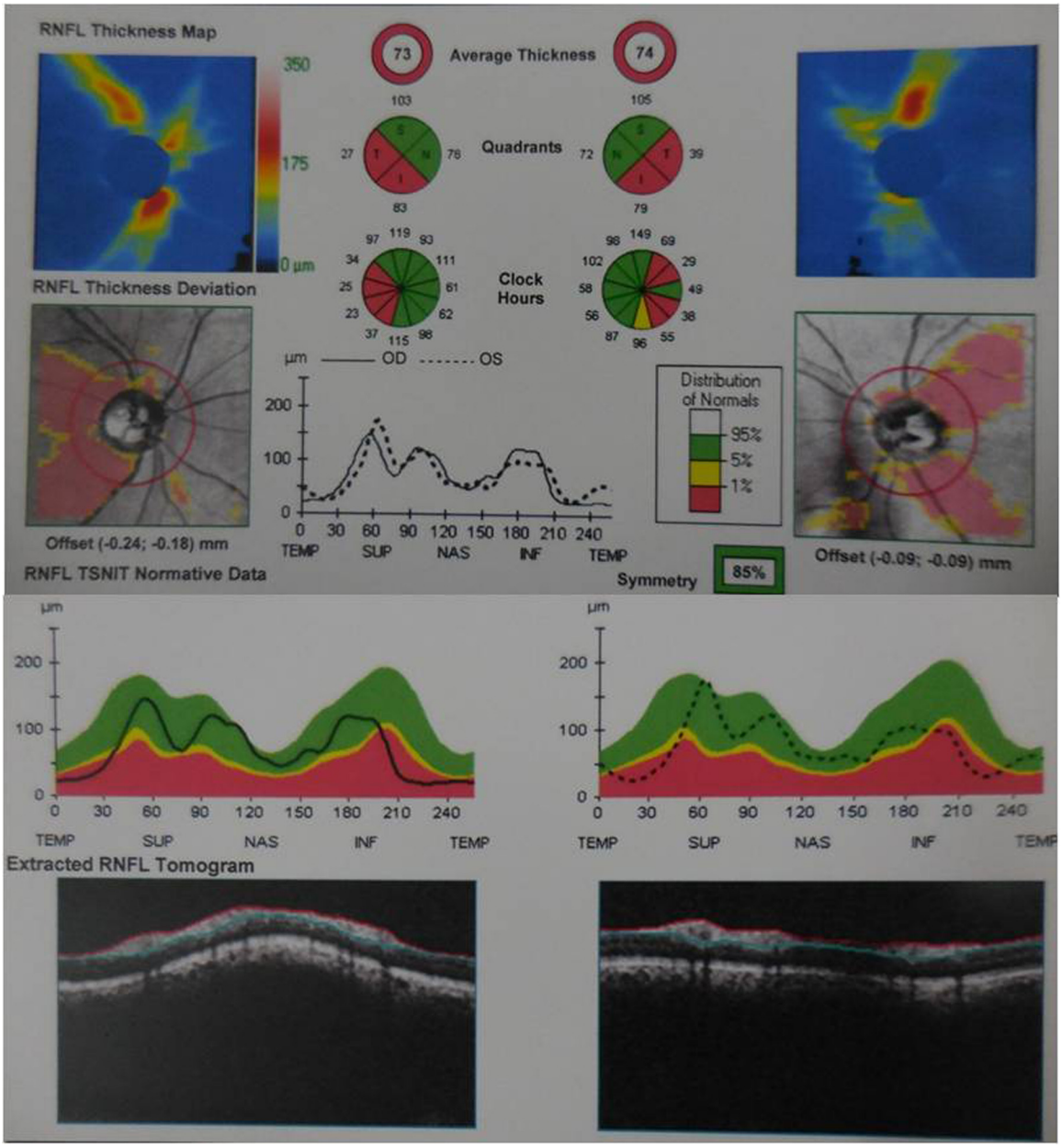

Fig. 4 – OCT analysis of retinal nerve fibre layer shows loss of temporal quadrant nerve fibres in both eyes.

toxicity is not yet known. Damage to the papillomacular bundle of the optic nerve due to inefficient mitochondrial functioning leading to axonal damage is thought to be the most likely mechanism.[3] When given for short durations, it is relatively well tolerated. Clinical trials have evaluated the safety of linezolid for up to 28 days.[4] In a case series of linezolid-induced peripheral neuropathy by Corallo and Paull,[5] one out of four patients developed optic neuropathy as well. The patient had been receiving the drug for 6 months for Methicillin Resistant Staphylococcus aureus (MRSA) prosthetic hip infection. Other serious side effects that need halting the drug include myelosuppression, and sometimes lactic acidosis and serotonin syndrome.[6]

Another drug known to cause optic neuritis is capreomycin.[7] In our study, the toxicity could not have been due to capreomycin, because the symptoms developed a month after stopping capreomycin for case 1, and case 2 was not on capreomycin at all. Isoniazid and ethambutol are the other antitubercular drugs implicated in optic toxicity. Though both patients were on high dose isoniazid, the drug was not suspected because toxicity due to isoniazid usually manifests early at the start of Anti-tubercular treatment (ATT), usually within 10 days.[8] Moreover, both patients improved after stopping linezolid. Optic neuritis due to tuberculosis itself is extremely rare.[8] Patients usually present with chorioretinitis, uveitis and miliary tuberculosis in such cases.

There is no known treatment of linezolid-induced neuropathy. Withdrawal of the drug leads to partial to complete resolution of visual symptoms but peripheral neuropathy is thought to be irreversible.[9] Although retinal nerve fibre layer

thinning remained in both our patients, visual symptoms completely resolved and patients remained asymptomatic on follow-up due to early recognition and timely withdrawal of the drug.

Linezolid is a bacteriostatic drug with unclear efficacy in the treatment of DR-TB. The rising incidence of XDR-TB along with the non-availability of effective drugs to fight the same has driven health professionals to devise a treatment with a bulk of less potent drugs for prolonged duration with unacceptable side effects. As many as 86% of patients on second line therapy develop drug side effects.[10] Regular monitoring is thus necessary to diagnose adverse drug reactions and take prompt actions.

5. Conclusion

Linezolid is one of the lesser-known drugs causing optic neuropathy. Physicians must be aware of the optic toxicity associated with linezolid and remain vigilant with regular ophthalmic evaluation for patients on long-term treatment with this drug.

Conflicts of interest

The authors have none to declare.

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Case Report

Isolated splenic tuberculosis diagnosed by endoscopic ultrasound-guided fine needle aspiration

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ABSTRACT

Our patient was a 48-year-old female, who presented with history of persistent low-grade fever and weight loss. The CT scan of the abdomen revealed multiple hypodense lesions in spleen. No primary focus of infection was detected in any other organs. Endoscopic ultrasound-guided fine needle aspiration of splenic lesion revealed granulomatous inflammation. The patient was started on anti-tuberculous therapy. There is a diagnostic possibility of splenic tuberculosis even in immunocompetent individuals and we chose a combination anti-tuberculous therapy as the first line treatment with consideration of splenectomy depending on the response.

A 48-year-old non-diabetic immunocompetent woman presented with a 2-month history of low-grade intermittent fever and weight loss. Complete haemogram showed normocytic normochromic anaemia and high erythrocyte sedimentation rate of 50 mm in first hour. The HIV serology was negative. The chest X-ray was within normal limits. Abdominal ultrasound showed moderately enlarged spleen with multiple ill-defined hypoechoic lesions. CT chest and abdomen was done which showed multiple hypodense lesions in spleen. Endoscopic ultrasound (EUS) showed multiple hypo-echoic lesions in the spleen as shown in Figs. 1 and 2 (FNA being done). EUS-guided fine-needle aspiration was done from one of the splenic lesions. The cytopathology showed epithelioid granulomas with areas of caseation in the centre surrounded by a variable number of Langhans giant cells, favouring the diagnosis of tuberculosis (Fig. 3). The patient was started on four-drug anti-tubercular treatment (HRZE). At follow-up after 2 months, her fever had responded gradually.

Splenic involvement in tuberculosis occurs commonly with other organs involvement.[1,2] However, isolated splenic tuberculosis is extremely rare and there is no uniform morphological appearance.[2] Differential diagnosis of multiple hypoechoic splenic lesions include tuberculosis, fungal infections, metastasis, lymphoma, and echinococcal lesions; hence, tissue diagnosis is important.[2] Due to proximity of spleen to gastric wall and real time visualisation of needle

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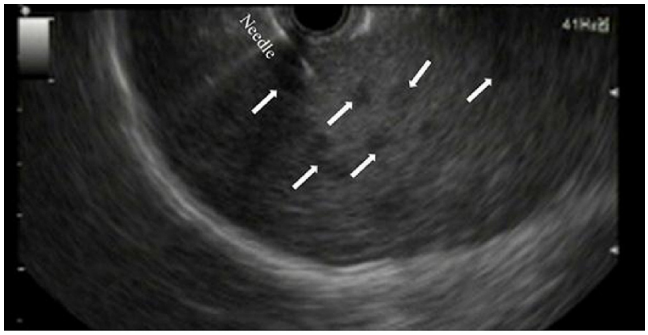

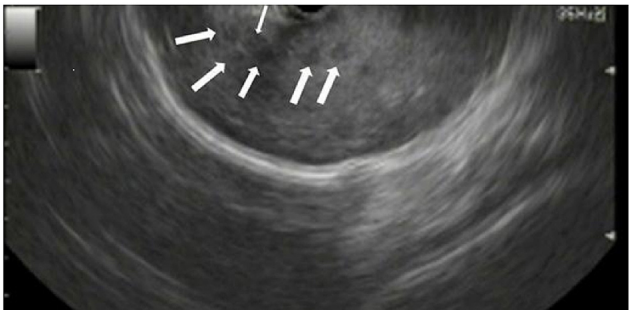

Figs. 1 and 2 – EUS images showing multiple hypo-echoic lesions in the spleen (arrow heads showing hypo-echoic lesions).

movement, EUS is an easy and important tool for FNA of splenic lesions and has been shown to be safe and effective even in patients with small lesions with failed ultrasound or CT-guided FNA attempts.[3,4] Majority of patients of isolated splenic tuberculosis are managed with anti-tubercular treatment.[5] Diffuse involvement of spleen with multiple nodules and non-response to AKT are indications for the early splenectomy.[6,7]

Conflicts of interest

The authors have none to declare.

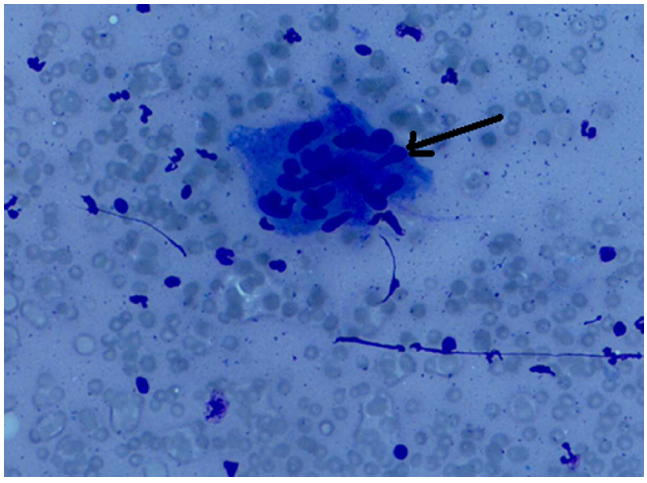

Fig. 3 – Photomicrograph shows caseous necrosis in the FNAB of the splenic abscess suggestive of tuberculous aetiology (H and E, ×10) (arrow head pointing towards granuloma).

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Case Report

Multifocal pure tubercular osteomyelitis: An unusual presentation in childhood

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ABSTRACT

Tuberculosis is a major health problem in the developing world. One-third of children infected with *Mycobacterium tuberculosis* have extra pulmonary involvement. Skeletal tuberculosis occurs in 1–6% of them with vertebra being the commonest site. Pure tubercular osteomyelitis without joint involvement occurs in only 2–3% cases of osteoarticular tuberculosis. Common sites are femur, tibia, and fibula. Disseminated skeletal involvement is very rare in children (7%) and calvarial osteomyelitis is even rarer (1%). Here, we report a unique case of disseminated skeletal TB. A 7-year-old tribal girl with no evidence of immunodeficiency presented with multiple lytic lesions involving skull, sternum, and hip bone surprisingly sparing the joints and appendicular skeleton. There was no pulmonary involvement either. FNAC from all three swellings showed presence of acid-fast bacillus. Bone biopsy followed by culture in BACTEC further confirmed the diagnosis. There was complete resolution of the swellings after one year of anti-tubercular drug therapy.

1. Introduction

Tuberculosis is a major health problem in the developing world with myriad presentations. WHO estimated half a million children were ill with tuberculosis worldwide in the year 2012 with the disease incidence decreasing very slowly.[1]

One-third of children infected with *Mycobacterium tuberculosis* have extra pulmonary involvement. Skeletal tuberculosis occurs in 1–6% of them.[2] Vertebra is the commonest site of involvement across all age groups.[3] Pure tubercular osteomyelitis without joint involvement occurs in only 2–3% cases of osteoarticular tuberculosis. Common sites are femur, tibia, and fibula.[4] Disseminated skeletal involvement is very rare in children (7%) and calvarial osteomyelitis is even rarer accounting for approximately 1% of skeletal tuberculosis disease.[5] We report a child with disseminated skeletal TB who presented to us with multiple lytic lesions involving skull, sternum, and hip bone.

2. Case report

A 7-year-old female patient from a tribal village of West Bengal presented with gradually increasing swelling over sternum,

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over right periorbital region and right lower back. The parents vaguely referred to them as being present for 1–2 years. All the swellings (Fig. 1a–c) were noticed around the same time.

The swelling over sternum was firm in consistency, nontender, not mobile, and fixed to underlying bone.

Swelling over right periorbital region was firm, nontender in relation to the zygomatic bone and was associated with a chronic discharging sinus over the lateral canthus of right eye. It caused misalignment of the two palpebral fissures and nonparalytic squint.

Swelling in right lower back was soft, fluctuating, nontender, and was in relation to right hip bone.

There was no history of trauma, persistent low-grade fever, cough, lymphadenopathy, weight loss, or contact with suspected or known case of TB.

On general examination, patient had pallor, discharging sinus over the lateral canthus of right eye and grade II PEM. No BCG scar was visible. No immunization records were available. Examination of respiratory system was normal and there was no lymphadenopathy or hepatoslenomegaly. Examinations of all other systems were unremarkable. Swelling in lumbar region was in relation to right hip bone but there was no restriction of movement at right hip joint.

Routine blood investigations were done. Complete hemogram: Hb% – 9gm%, TC 11 $\times$ 10^9/L, PLT – 4 $\times$ 10^{11}/L. Peripheral blood film showed evidence of anemia of chronic disorder. ESR was 92 mm in first hour LFT and RFT were within normal range. HIV ELISA was negative.

Mantoux test was positive (12 mm $\times$ 14 mm). X-ray chest revealed no lung parenchymal lesion; Skeletal X-rays revealed multifocal bone destruction in the calvarium, sternum, and right hip bone. USG showed hypoechoic globular swelling (3.45 cm $\times$ 3 cm) in the sternum and elongated SOL (5.7 cm $\times$ 2 cm) with internal fluid, in the right hip bone. No effusion was found in right hip joint. USG guided FNAC of all three swellings and sinus tract revealed presence of acid-fast bacilli.

Fig. 1 – (a) Note the swellings at the manubrium sterni and right periorbital region. Chronic discharging sinus over the lateral canthus of right eye. It caused misalignment of the two palpebral fissures and nonparalytic squint. (b) Right lateral view showing swelling in the right periorbital region in relation to the zygomatic bone and the discharging sinus over right lateral canthus. (c) Swelling in right lower back was soft, fluctuating, nontender, and was in relation to right hip bone.

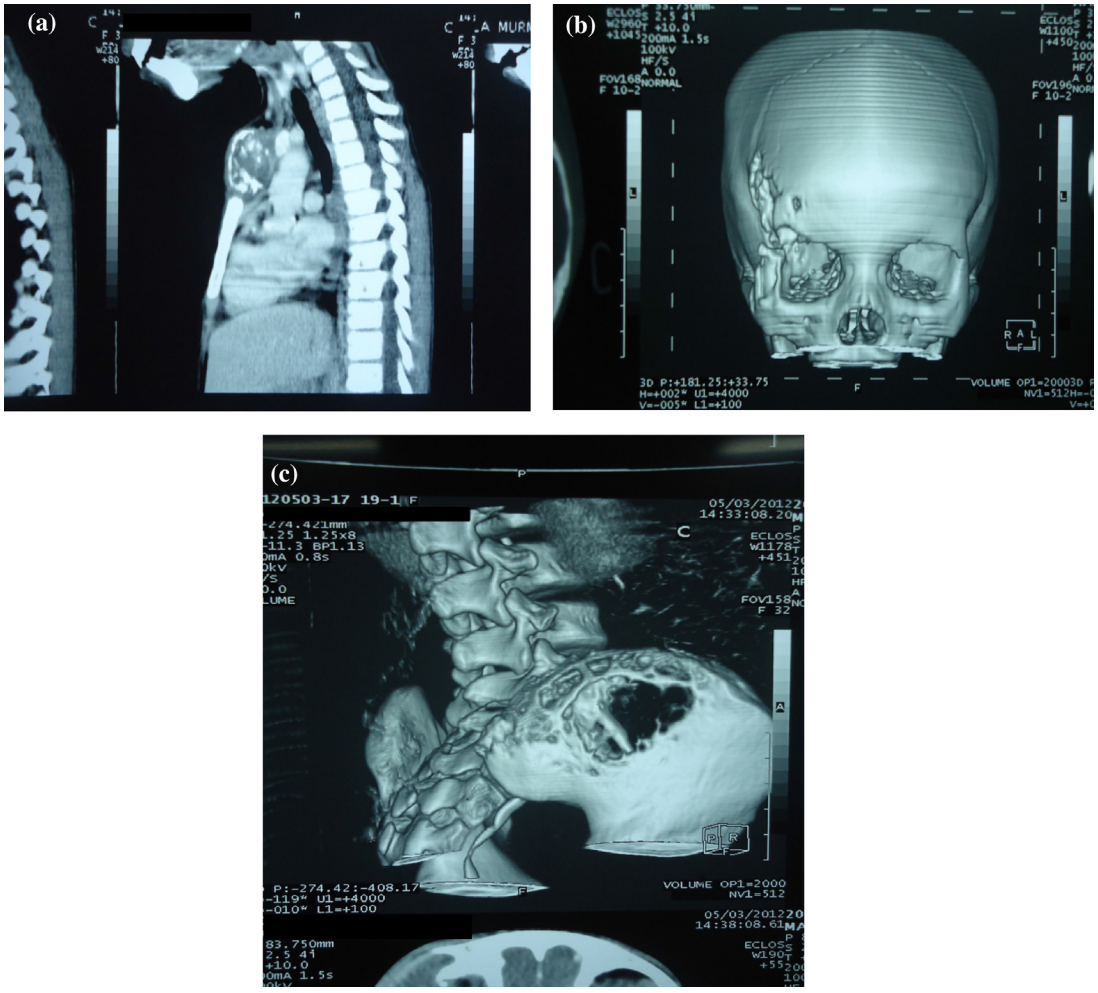

Fig. 2 – (a) Reconstructed CT images showing Gross destruction with osteolysis at the manubrium sternum with overlying soft tissue thickening. Destruction is more at the inner cortex with extension of soft tissue component at the superior mediastinum. (b) Reconstructed CT images showing small lytic area at the right side of frontal bone which extends to involve the zygomatic bone near superior orbital margin. (c) Reconstructed CT image showing multiple lytic lesions with conglomerations and irregular margin at right iliac blade.

CT of all three areas showed massive destruction of underlying bone (Fig. 2a–c). In the skull, the floor and lateral wall of the orbit with parts of frontal bone in the right side was involved. The manubrium sterni showed complete osteolysis. In the right hip bone, major part of the ilium was destroyed, but hip joint was not involved.

Bone biopsy from hip bone revealed caseous necrosis with epitheliod granulomas and multiple giant cells. Aspirates as well as bone biopsy were sent for culture in BACTEC which revealed *M. tuberculosis* growth.

Sputum examination was negative for acid-fast bacilli on three consecutive days.

Bone marrow aspiration was unremarkable. Fungal blood cultures with special reference to histoplasmosis did not show any growth. Serum protein electrophoresis did not reveal any abnormal bands.

The patient was treated with anti-tubercular drugs for one year. Three months intensive phase with four drugs (HRZE) followed by nine months of maintenance phase with two drugs (HR) under DOTS, RNTCP was prescribed. There was complete resolution of swellings and healing of the sinus tract.

3. Discussion

Osteoarticular involvement occurs in 1–6% of patients with extrapulmonary tuberculosis. Skeletal tuberculosis is common in spine and large joints like hip and knee secondary to lymphohematogenous spread of *M. tuberculosis* from a pulmonary focus with absent radiographic evidence of pulmonary involvement in 50% cases.[6]

Other causes include direct spread from overlying soft tissue structures or lymph nodes and rarely after BCG vaccination.

The commonly affected sites in TB osteomyelitis are spine accounting for 50% of cases followed by femur, tibia, and fibula in that order of frequency. Pure tubercular osteomyelitis without joint involvement is rare. Disseminated skeletal

tuberculosis in an immunocompetent host is also rare. Thus, the present case is unique in a number of ways: firstly, pure tubercular osteomyelitis was present; secondly, it was multifocal; thirdly, only flat bones of the axial skeleton involved, no pulmonary involvement was found, and finally host was immunocompetent. Very few cases of multifocal tubercular osteomyelitis without pulmonary involvement are reported in literature, and in all those cases either spine or the long bones were involved.

Tuberculosis of sternum is rare with few reported cases. Out of these, majority is in adult, immunocompromised patients. However, commonest site is the manubrium as in our case.

TB skull is very rare accounting for less than 1% cases of tubercular osteomyelitis. Only five to six cases were reported. Frontal and parietal bones were most commonly involved because of the high cancellous bone content. In our patient, outer table of the frontal bone was involved with extension to the right zygomatic bone and sinus formation.

Our patient had TB of the hip bone without involvement of the hip joint but with soft tissue extension. There was conglomeration of multiple osteolytic lesions as seen in the CT images. No such involvement is reported in literature.

Differential diagnosis includes chronic pyogenic osteomyelitis, primary bone tumor, secondary metastasis, granulomatous diseases, inflammatory arthritis and sarcoma, eosinophilic granuloma, disseminated lymphangiomatosis, and fungal infections such as histoplasmosis or cryptococosis.

Diagnosis of TB osteomyelitis requires a high index of clinical suspicion. Tuberculosis of the bone can go unnoticed for a long time until there is extension of the disease to skin and adjacent structures including the joints. Localized pain and swelling are relatively common early clinical manifestations. Cutaneous stigmata such as chronic discharging sinuses seen in our patient are late manifestations. Clinical symptoms are very nonspecific and can include insidious onset of pain, swelling, decreased range of motion, and difficulty ambulating. Patients may also have weight loss, night sweats, generalized malaise, and decreased appetite.[7,8] Constitutional symptoms are uncommon unless there is concomitant pulmonary disease, as in our case. Pulmonary disease may not be evident in young children and in endemic areas.

Radiographic appearances of TB osteomyelitis depend on the stage of presentation at diagnosis, ranging from mild soft tissue swelling to areas of osteolysis. Infection starts from cancellous bony segments. Imaging studies characteristically show osteopenia and rare sequestration or periosteal reaction thus differentiating it from pyogenic osteomyelitis.[7–9] CT, MRI or myelography may be more informative.

A positive tuberculin skin test is an important clue in diagnosing tuberculosis, but is negative in 10% of the patients. ESR can be elevated, but is often normal.[10,11] In our patient, ESR was 92 mm. The gold standard for diagnosis is the isolation of *Mycobacterium tuberculosis* from cultures of bone biopsy material. Histopathology of the bone lesions also is very helpful in confirming the diagnosis[7,8,12] Diagnosis was confirmed by demonstration of epithelioid granulomas and acid-fast bacilli and a positive *M. tuberculosis* culture from the aspirate taken from the swellings. Extensive diagnostic work-up did not reveal any other focus of tuberculosis. A significant challenge in the diagnosis of tubercular osteomyelitis is that the smears for acid-fast bacilli are often negative, leading to a delay in diagnosis while waiting for the organisms to grow in culture media.[13,14] Polymerase chain reaction or nucleic amplification assays may be helpful in obtaining an earlier diagnosis; however, a negative result does not rule out tuberculosis.

4. Conclusion

Clinical and radiographic presentation of skeletal tuberculosis in endemic area differs from non-endemic area, with a higher incidence of multifocal involvement and severe bone destruction in endemic area. Thus, a high index of suspicion leads to earlier detection of cases and prevents dissemination in this essentially treatable disease.

Conflicts of interest

The authors have none to declare.

Author contributions

S. Pati and S. De were involved in history taking, patient management and preparation of manuscript, T.N. Ghosh and M.K. Ghosh diagnosed the case and provided overall guidance.

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Case Report

Spontaneous chylothorax revealing a mediastinal and abdominal lymph node tuberculosis

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ABSTRACT

Chylothorax is a rare manifestation of tuberculosis. We report a case of spontaneous chylothorax due to tuberculosis. A 62-year-old woman was admitted with fever, chest pain and dyspnea. Chest and abdominal computed tomography revealed a fluid collection with necrotic mediastinal and abdominal lymph nodes. Biopsy of lymph nodes by mediastinoscopy. The patient was treated with anti-tuberculosis medication. He is clinically improved and his pleural effusion also completely resolved.

1. Introduction

Chylothorax, an uncommon cause of pleural effusion, results from the accumulation of lymph in the pleural space due to damage or obstruction of the thoracic duct.

The high content of triglycerides and the presence of chylomicrons set the diagnosis of chylothorax. Malignancy and trauma are the leading causes of chylothorax. Lymph node tuberculosis is an exceptional etiology of chylothorax.[1,2]

We report a case of chylothorax due to lymph nodes tuberculosis.

2. Case report

A 62-year-old woman was admitted to our department with a one-month history of fever, right-sided chest pain, non-productive cough and weight loss of 6 kg. She did not give any history of trauma. On admission, body temperature was 38.5 °C, heart rate was 80/min and body weight was 70 kg. On chest auscultation, breathing sound was decreased on right lower lung. There were no palpable lymphadenopathies. Abdominal examination was normal. A chest radiograph taken on admission showed right pleural effusion (Fig. 1).

Initial laboratory results were as follows: white blood cell count 8900/mm^3, hemoglobin 14 g/dL, serum total protein 5.9 g/dL, albumin 2.69 g/dL and elevated erythrocyte sedimentation rate of 35 mm in half hour. Renal and liver function tests were within normal limits. Sputum smear examination for acid-fast bacilli was negative in the three samples obtained.

The pleural fluid was aspirated, revealed a milky white fluid.

The pleural fluid was sent for examination that revealed, protein 75 g/l and total leukocyte count 5500 cells/mm^3; differential leukocyte count was neutrophils 5%, lymphocytes 95%, pleural fluid triglyceride 56.09 mmol/l (49.64 g/l) and pleural fluid cholesterol 3, 47 mmol/l (1.34 g/l). Serum triglyceride and serum cholesterol was 0.56 mmol/l and 3.27 mmol/l,

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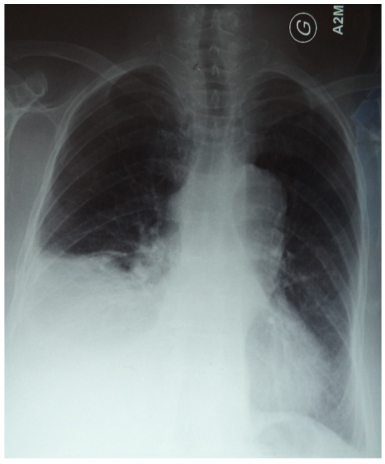

Fig. 1 – Chest X-ray on admission: right pleural effusion.

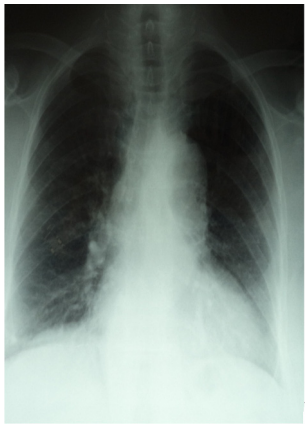

Fig. 3 – Chest X-ray after treatment.

respectively. The Ziehl–Neelsen stain of the pleural fluid was negative and also pleural fluid culture for pyogenic organisms was sterile. Pleural fluid cytology was negative for malignant cells.

Chest and abdominal computed tomography (CT) showed right pleural effusion with necrotic mediastinal and abdominal lymph nodes (Fig. 2).

Biopsy of lymph nodes by mediastinoscopy revealed caseating granuloma, and the Bactec culture for *M. tuberculosis* was also positive in the mediastinal lymph node biopsy specimen.

We diagnosed the patient as having lymph nodes TB associated with chylothorax.

The patient received anti-TB treatment with standard 6-month antitubercular treatment: a combination of isoniazid, rifampicin, pyrazinamide, and ethambutol was started for 2 months followed by isoniazid and rifampicin for a further 4 months. The patient also was put on a high protein and low fat diet with medium chain triglyceride. Following this, she showed clinical as well as radiological improvement and chylothorax resolved after treatment, and on regular follow-up she had no further symptoms (Fig. 3).

3. Discussion

Chylothorax is characterized by chyle in the pleural cavities produced by obstruction and disruption of the lymphatic channel. The etiologies of chylothorax can be nontraumatic and traumatic. The most common cause of nontraumatic chylous effusion is a malignancy, such as lymphoma (75% cases) or metastatic carcinoma[1,2] and they are related to tumor invading the thoracic lymph duct.

Other causes of nontraumatic chylous effusion include idiopathic, congenital anomaly, protein-losing enteropathy and tuberculosis.[3]

Trauma is the second leading cause of chylothorax, responsible for 25% of cases. Surgery is the most common cause of traumatic chylothorax, especially in operations that mobilize the left subclavian artery.

The exact pathogenesis for the development of chylothorax secondary to tuberculosis remains controversial. The enlarged lumber and iliac group of lymph nodes produced obstruction of the cisterna chyli and thoracic duct, as a result of which there was dilatation of the lumbar channels; this was followed by the opening up of collateral anastomoses, many

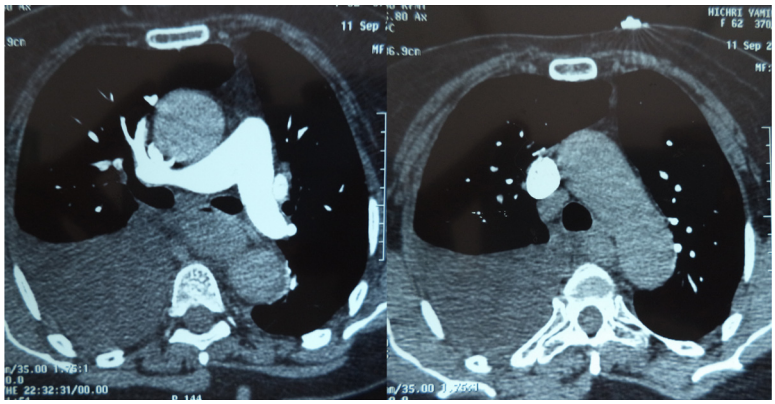

Fig. 2 – Computed tomography of chest showed a large lymphadenopathy in mediastinum.

lymphaticovenous anastomoses existing between the thoracic duct system and the azygos, intercostal, and lumbar veins. The increased pressure in the system resulted in the transudation of chyle into the pleural space. Grobbelaar et al. reported that the possible explanation for the development of a chylothorax is the obstruction of the thoracic duct by tuberculous lymphadenopathy with subsequent increase in pressure in the surrounding lymphatic system and leaking of chyle into the pleural space.[4]

In our patient, chylothorax developed possibly due to the enlargement of mediastinal lymph nodes, which obstructed the thoracic duct flow and resulted in chyle leakage into the pleural space.

The mainstay of the treatment of chylothorax is conservative measures and correcting the underlying causes. The patient received anti-TB medication and nutrition replacement with high protein and low fat meal with medium chain triglycerides.

Although some case reports have described the use of octreotide in the management of chylothorax or chylous ascites.[5]

Conflicts of interest

The authors have none to declare.

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Case Report

Anaphylaxis to rifampicin and pyrazinamide in a child with tuberculous meningitis: A case report

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ABSTRACT

Rifampicin (RFP) and pyrazinamide (PZA) are the primary anti-tubercular drugs with a considerably safe profile. However, none of the drugs are without adverse reactions. They both can lead to a variety of adverse effects including life-threatening anaphylaxis. We report an interesting and possibly the first case of concurrent hypersensitivity to two primary anti-tubercular treatment (ATT) drugs. Hypersensitivity to RFP and PZA was confirmed in this patient by drug provocation and intradermal skin testing. He improved on alternative ATT regime withdrawing RFP and PZA.

1. Introduction

Anti-tubercular treatment (ATT) drugs are relatively safe, and serious adverse events such as life threatening anaphylaxis are reported uncommonly. We report a case of rifampicin (RFP) and pyrazinamide (PZA) induced anaphylaxis in a 12-year-old boy who was under treatment for tuberculous meningitis. Anaphylaxis was managed, and both the drugs were withdrawn. Subsequently the child was put on isoniazid, ethambutol, and streptomycin during the intensive phase, followed by isoniazid and ethambutol. On last follow-up, 3 months back, the patient completed 13 months of therapy and was improving without any further complications.

2. Case report

A 12-year-old male child under treatment for tuberculous meningitis for 12 days presented to the outpatient department with mild fever and generalized itching. He was on four-drug ATT, RFP, isoniazid, ethambutol and PZA along with oral prednisolone and phenytoin. Since phenytoin is most commonly associated with skin rashes and allergy,[1] it was stopped and phenobarbitone was started instead. Three days later, the child was brought to the emergency department in a state of shock. He was managed with fluid boluses, Inj. adrenaline, Inj. hydrocortisone and antihistaminics. All the drugs were stopped.

Five days later, ATT was gradually restarted. Isoniazid was introduced first. Five days later, Ethambutol was added. This was also tolerated without any side effects. Subsequently when RFP was added, within an hour of ingestion, the child had generalized itching and full-blown picture of anaphylaxis, which was managed appropriately. Two weeks later, the child was administered PZA in the hospital. Two hours later, he had a similar prodrome with anaphylactic reaction. In view of severe allergic reaction, both these drugs were withdrawn and Inj. streptomycin was instead added to the regime. The patient was discharged on oral isoniazid, and ethambutol with Inj. streptomycin.

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To establish hypersensitivity, intradermal skin test was performed after taking a written consent from the parents. A method similar to Kim et al.[2] was used. For intra-individual comparison, histamine (0.01%, as positive control) and 0.9% normal saline (as negative control) were tested along with the offending drug by intradermal administration. For inter-individual comparison, four healthy controls were taken and subjected to the same test as our patient. RFP was diluted in normal saline in increasing concentrations starting from 0.1 mg/ml to 1 mg/ml. The child developed a wheal and flare, with an induration measuring 15 mm × 12 mm to intradermal administration of 0.1 ml of 0.1 mg/ml concentration. Similar strength of RFP was also tested on four healthy controls; however, there was no reaction even after 20 min. Hypersensitivity to PZA was tested by intradermal administration of 0.1 ml at a strength of 0.1 mg/ml, which led to an induration of 15 mm × 15 mm. None of the controls had any reaction to PZA also. Local anergy was ruled out by a significant reaction with injection of positive and negative control in the patient. Hypersensitivity to both RFP and PZA was thus established by the oral challenge and the intradermal tests in our patient.

The child received three drug ATT, streptomycin, isoniazid and ethambutol during the intensive phase. Streptomycin was stopped after 90 days. At present, he is on isoniazid and ethambutol and is recovering well. Since the patient has been recovering well with modified ATT regime, we intend treating him for at least 18 months as both the drugs mandatorily needed for a short course chemotherapy had to be withdrawn due to severe reaction.

3. Discussion

RFP, an effective anti tubercular drug, is usually tolerated well without any adverse reactions. However many drug reactions like fever, rash, flu-like syndrome, hemolytic anemia, thrombocytopenia have been mentioned.[3] Anaphylaxis, a lesser known entity, has rarely been reported.[4–6] To the best of our knowledge, no case has so far been reported from India. It has been reported previously due to isoniazid.[7] Pathogenesis of anaphylaxis due to RFP has not been completely elucidated but seems to be IgE mediated. Exposure to RFP leads to formation of IgE antibodies with facilitation by Th2 subtype of CD4 lymphocytes. These IgE antibodies then bind to the Fc-E receptor of basophils and monocytes. On re-exposure, the IgE-RFP complex leads to activation of these cells. Following activation, various soluble mediators of anaphylaxis such as histamine, proteases, leukotrienes, and prostaglandins are released thereby causing an anaphylactic reaction.[6]

The intermittent regimen in comparison to the continuous one has longer interval to accumulate more RFP antibodies; hence in such cases, more immunogenic side effects are seen on re-exposure to RFP.[4,6] Review of literature revealed that the interval between the start of treatment and the appearance of the adverse reaction (anaphylaxis) is highly variable ranging from the first dose to 20 months,[6] being 12 days in our case and in most of the cases these reaction appeared soon after re-administration of RFP. Most of the patients presented with prodromes before the anaphylaxis reaction, rash being the most common[6] as was seen in our case too.

Anaphylaxis due to PZA was also recently reported by Bavbek et al.[8] Although a definite pathogenesis of PZA induced anaphylaxis cannot be firmly concluded, but in the light of present evidences, IgE mediated reaction can be hypothesized for PZA[8] as proposed for RFP.[2]

There have been reports where anti-RFP IgE antibodies have been detected by radioallergosorbent test (RAST) in patients exhibiting anaphylactic reactions. RFP-dependent IgG and IgM antibodies can also be tested in the serum.[9] Immunological investigations for confirmation of such reactions is a zone which still needs to be worked on. There have been reports where continuation of the offending drug was possible after desensitization.[8,10] We did not try desensitization in our case and instead chose alternative regime in our patient who is responding to the same.

4. Conclusion

RFP and PZA are important primary ATT drugs. Though they are considered drugs with a relatively safe profile, the above case highlights that the possibility of a severe anaphylactic reaction should always be kept in mind. The treating pediatrician should always get alarmed in a patient who presents even with a minor rash while on ATT as it could be harbinger of a major trouble later on. Co-existence of hypersensitivity to two primary anti-tubercular drugs is possibly unreported till now.

Conflicts of interest

The authors have none to declare.

Ethical approval

Informed written consent was obtained from the parents for the intradermal test and publication of the case report.

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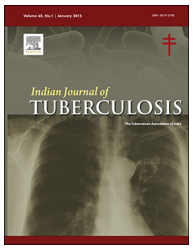

Abstract

Edge map analysis in chest X-rays for automatic pulmonary abnormality screening

Santosh KC, Vajda S, Antani S, et al. *Int J CARS*. (2016);11:1637. http://dx.doi.org/10.1007/s11548-016-1359-6

Purpose. Our particular motivator is the need for screening HIV$^+$ populations in resource-constrained regions for the evidence of **Tuberculosis**, using posteroanterior chest radiographs (CXRs).
Method. The proposed method is motivated by the observation that abnormal CXRs tend to exhibit corrupted and/or deformed thoracic edge maps. Texture changes in the lung sections (due to abnormalities) can be represented by their corresponding edge maps. We study histograms of thoracic edges for all possible orientations of gradients in the range [0, 2π) at different numbers of bins and different pyramid levels, using five different regions-of-interest selection.
Results. We have used two CXR benchmark collections made available by the U.S. National Library of Medicine and have achieved a maximum abnormality detection accuracy (ACC) of 86.36% and area under the ROC curve (AUC) of 0.93 at 1 s per image, on average.
Conclusion. We have presented an automatic method for screening pulmonary abnormalities using thoracic edge map in CXR images. The proposed method outperforms previously reported state-of-the-art results. In particular, it outperforms Jaeger et al. 2014, doi: 10.1109/TMI.2013.2284099 by more than 3% in AUC, and the tool is 25 times faster. This shows the interest in mass screening. Therefore, to be considered in Kenya, South Africa (URL: https://lhncbc.nlm.nih.gov/project/computer-aided-tb-screening-chest-x-rays), this tool is appropriate and justified.
Keywords: Automation; Thoracic edge map; Pulmonary abnormalities; Screening; Tuberculosis; Chest X-rays.
Funding: This research was supported by the Intramural Research Program of the National Institutes of Health (NIH), National Library of Medicine (NLM), and Lister Hill National Center for Biomedical Communications (LHNCBC).
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Abstracts

Examples of bedaquiline introduction for the management of multidrug-resistant tuberculosis in five countries

Guglielmetti L, Hewison C, Avaliani Z, Hughes J, Kiria N, Lomtadze N, Ndjeka N, Setkina S, Shabangu A, Sikhondze W, Skrahina A, Veziris N, Furin J. *Int J Tuberc Lung Dis*. 2017;21 (2):167–74. https://doi.org/10.5588/ijtld.16.0493.

Background: For the first time in almost 50 years, there are new drugs available for the treatment of tuberculosis (TB), including bedaquiline (BDQ) and delamanid (DLM). The rate of introduction, however, has not kept pace with patient needs. It is estimated that as many as 23% of multidrug-resistant TB (MDR-TB) patients have an indication for receiving BDQ. As this is the first time the MDR-TB community is introducing new medications, it is important to understand how implementation can be developed in a variety of settings.

Methods: A qualitative assessment of country TB programs in which more than 5% of MDR-TB patients were started on BDQ under program conditions.

Results: National TB programs in Belarus, France, Georgia, South Africa, and Swaziland all started sizeable cohorts of patients on BDQ in 2015. Common factors observed in these programs included experience with compassionate use/expanded access, support from implementing partners, and adequate national or donor-supported budgets. Barriers to introduction included restriction of BDQ to the in-patient setting, lack of access to companion drugs, and the development of systems for pharmacovigilance.

Conclusion: The five countries in this paper are examples of the introduction of new therapeutic options for the treatment of TB.

Conflicts of interest

The authors have none to declare.

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Mycobacterium tuberculosis Rv1474c is a TetR-like transcriptional repressor that regulates aconitase, an essential enzyme and RNA-binding protein, in an iron-responsive manner

Balakrishnan K, Mohareer K, Banerjee S. *Tuberculosis*. 2017;102 (January). http://dx.doi.org/10.1016/j.tube.2017.01.003.

Mycobacterium tuberculosis (*M.tb*), tuberculosis (TB) causing bacteria, employs several mechanisms to maintain iron homeostasis which is critical for its survival and pathogenesis. *M.tb* aconitase (Acn), a [4Fe-4S] cluster-containing essential protein, apart from participating in energy cycle, also binds to predicted iron-responsive RNA. In this study, we identified Rv1474c as a TetR-like repressor of its operonic partner *acn* and carried out its biochemical and functional characterization. The binding motif for Rv1474c in the upstream region of *acn (Rv1475c)-Rv1474c* operon was verified by gel-shift assays. Reporter assays in *Escherichia coli* followed by over-expression studies in mycobacteria, using both wild type and a DNA-binding defective mutant, demonstrated Rv1474c as a Tet-R like repressor of *acn*. Rv1474c, beside binding tetracycline, could also bind iron which negatively influenced its DNA binding activity. A consistent decrease in the relative transcript levels of *acn* when *M.tb* was grown in iron-deficient conditions as compared to either normal or other stress conditions, indicated regulation of *acn* by Rv1474c in an iron-responsive manner *in vivo*. The absence of homologs in the human host and its association with indispensable iron homeostasis makes Rv1474c an attractive target for designing novel anti-mycobacterials.

Conflicts of interest

The authors have none to declare.

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Factors influencing treatment default among tuberculosis patients in a high burden province of South Africa

Kigozi G, Heunis C, Chikobvu P, Botha S, van Rensburg D. *Int J Infect Dis*. 2017;54(January):95–102. http://dx.doi.org/10.1016/j.ijid.2016.11.407.

Objective: To determine and describe the factors influencing treatment default of tuberculosis (TB) patients in the Free State Province of South Africa.

Methods: A retrospective records review of pulmonary TB cases captured in the ETR.Net electronic TB register between 2003 and 2012 was performed. Subjects were >15 years of age and had a recorded pre-treatment smear result. The demographic and clinical characteristics of defaulters were described. Multivariate logistic regression analysis was used to determine factors associated with treatment default.

The odds ratios (OR) together with their corresponding 95% confidence intervals (CI) were estimated. Statistical significance was considered at 0.05.
Results: A total of 7980 out of 110 349 (7.2%) cases defaulted treatment. Significantly higher proportions of cases were male (8.3% vs. female: 5.8%; $p < 0.001$), <25 years old (9.1% vs. 25–34 years: 8.7%; 35–44 years: 7.0%; 45–54 years: 5.2%; 55–64 years: 4.4%; >64 years: 3.9%; $p < 0.001$), undergoing TB retreatment (11.0% vs. new cases: 6.3%; $p < 0.001$), had a negative pre-treatment sputum smear result (7.8% vs. positive smear results: 7.1%; $p < 0.001$), were in the first 2 months of treatment (95.5% vs. >2 months: 4.8%; $p < 0.001$), and had unknown HIV status (7.8% vs. HIV-positive: 7.0% and HIV-negative: 5.7%; $p < 0.001$). After controlling for potential confounders, multivariate analysis revealed a two-fold increased risk of defaulting treatment when being retreated compared to being treated for the first time for TB (adjusted OR (AOR) 2.0, 95% CI 1.85–2.25). Female cases were 40% less likely to default treatment compared to their male counterparts (AOR 0.6, 95% CI 0.51–0.71). Treatment default was less likely among cases >24 years old compared to younger cases (25–34 years: AOR 0.8, 95% CI 0.77–0.87; 35–44 years: AOR 0.6, 95% CI 0.50–0.64; 45–54 years: AOR 0.4, 95% CI 0.32–0.49; 55–64 years: AOR 0.3, 95% CI 0.21–0.43; >64 years: AOR 0.3, 95% CI 0.19–0.35). Co-infected cases receiving antiretroviral therapy (ART) were 40% less likely to default TB treatment relative to those whose ART status was unknown (AOR 0.6, 95% CI 0.46–0.57).
Conclusions: Salient factors influence TB patient treatment default in the Free State Province. Therefore, the strengthening of clinical and programmatic interventions for patients at high risk of treatment default is recommended. In particular, ART provision to co-infected cases facilitates TB treatment adherence and outcomes.

Conflicts of interest

The authors have none to declare.

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Paradoxical results of two automated real-time PCR assays in the diagnosis of pleural tuberculosis

Morales-López SE, Yepes JA, Anzola I, Aponte H, Llerena-Polo CR. *Int J Infect Dis*. 2017;54(January):36–8. http://dx.doi.org/10.1016/j.ijid.2016.10.025.
Tuberculosis (TB) is a major cause of worldwide mortality. We report the case of a non-HIV-infected woman with clinical suspicion of pleural tuberculosis and contradictory results between Xpert® MTB/RIF and Abbott RealTime MTB assays from pleural fluid specimen. Liquid and solid cultures for tuberculosis were performed with negative results. The patient received treatment, and clinical improvement was observed. Both techniques detect *Mycobacterium tuberculosis* complex, but they have different targets and limits of detection. Abbott RealTime MTB results correlated well with the clinical findings of the patient.

Conflicts of interest

The authors have none to declare.

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What a difference a day makes: Same-day vs. 2-day sputum smear microscopy for diagnosing tuberculosis

Deka DJ, Choudhury B, Talukdar P, Lo TQ, Das B, Nair SA, Moonan PK, Kumar AMV. *Public Health Action*. 2016;6(December(4)):232–6. https://doi.org/10.5588/pha.16.0062.
Setting: Nine district-level microscopy centres in Assam and Tripura, India.
Objective: Same-day sputum microscopy is now recommended for tuberculosis (TB) diagnosis. We compared this method against the conventional 2-day approach in routine programmatic settings.
Methods: During October–December 2012, all adult presumptive TB patients were requested to provide three sputum samples (one at the initial visit, the second 1 h after the first sample, and the third the next morning) for examination by Ziehl–Neelsen smear microscopy. Detection of acid-fast bacilli with any sample was diagnostic. The first and second spot sample comprised the same-day approach, and the first spot sample and next-day sample comprised the 2-day approach.
Results: Of 2168 presumptive TB patients, 403 (18.6%) were smear-positive according to the same-day method compared to 427 (19.7%) by the 2-day method (McNemar's test, $P < 0.001$). Of the total 429 TB patients, 26 (6.1%) were missed by the same-day method and 2 (0.5%) by the 2-day method.
Conclusion: Same-day specimen collection for microscopy missed more TB than 2-day collection. In India, missing cases by using same-day microscopy would translate into a considerable absolute number, hindering TB control efforts. We question the indiscriminate switch to same-day diagnosis in settings where patients reliably return for testing the next day.

Conflicts of interest

The authors have none to declare.

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MDR-TB in Puducherry, India: Reduction in attrition and turnaround time in the diagnosis and treatment pathway

Shewade HD, Govindarajan S, Thekkur P, Palanivel C, Muthaiah M, Kumar AMV, Gupta V, Sharath BN, Tripathy JP, Vivekananda K, Roy G. *Public Health Action*. 2016;6(December (4)):242–6. https://doi.org/10.5588/pha.16.0075.
Setting: A mixed-methods operational research (OR) study was conducted to examine the diagnosis and treatment pathway of patients with presumptive multidrug-resistant tuberculosis (MDR-TB) during 2012–2013 under the national TB programme in Puducherry, India. High pre-diagnosis and pre-treatment attrition and the reasons for these were identified. The recommendations from this OR were implemented and we planned to assess systematically whether there were any improvements.
Objectives: Among patients with presumptive MDR-TB (July–December 2014), (1) to determine pre-diagnosis and pre-treatment attrition, (2) to determine factors associated with pre-diagnosis attrition, (3) to determine the turnaround time (TAT) from eligibility to testing and from diagnosis to treatment initiation, and (4) to compare these findings with those of the previous study (2012–2013).
Design: This was a retrospective cohort study based on record review.

Results: Compared to the previous study, there was a decrease in pre-diagnosis attrition from 45% to 24% ($P < 0.001$), in pre-treatment attrition from 29% to 0% ($P = 0.18$), in the TAT from eligibility to testing from a median of 11 days to 10 days ($P = 0.89$) and in the TAT from diagnosis to treatment initiation from a median of 38 days to 19 days ($P = 0.04$). There is further scope for reducing pre-diagnosis attrition by addressing the high risk of patients with human immunodeficiency virus and TB co-infection or those with extra-pulmonary TB not undergoing drug susceptibility testing.

Conclusion: The implementation of findings from OR resulted in improved programme outcomes.

Conflicts of interest

The authors have none to declare.

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