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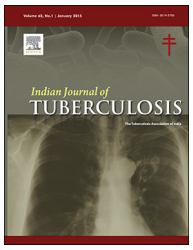

Editorial

TB treatment options: Beyond DOTS

Even though the availability of rapid and advanced diagnostic technology and an effective treatment of tuberculosis were discovered, this disease still remains a part of global epidemiological scenario. The economic burden of TB is extremely high. To achieve the goal of TB elimination by 2025 (as per new strategic plan) Ministry of Health and Family Welfare (MOHFW), Government of India has developed some strategies which are to be adopted apart from DOTS. In these strategies proper counselling of patients is of utmost importance not only for treatment success but also from the programme's point of view. The ground level staff has to be trained with 10 point TB/drug resistant TB counselling tool which covers various aspects of the disease. Although the tool is available but its sensitization among field workers is still lacking.

Latest Technical and Operational Guidelines (TOG) for TB control are available on the *tbcindia* website. This document covers strategies and guidelines for diagnosis and treatment of TB, programme management aspects covering patient support systems, human resource management, partnerships for TB control and ACSM activities. These guidelines should be widely circulated across all health providers engaged in TB control.[1]

New Strategic Plan (NSP) for TB elimination 2017–25 is a framework to guide the activities of all stakeholders, is a 3-year plan and an 8-year strategy document which provides goals and strategies for the country's response to the disease during the period 2017–2025. It draws attention on the most important interventions that the RNTCP believes will bring about significant changes in the incidence, prevalence and mortality of TB. These strategies and interventions are in addition to the processes and activities already ongoing in the country. NSP replaces previous strategies and will inform and guide the technical and operational guidelines refresh and associated programme tools modifications.[2]

Some of the salient indicators to be achieved by 2025 are: reduce estimated TB incidence rate (per 100,000) i.e. 44/lakh/year by 2015; reduce estimated TB prevalence rate (per 100,000) i.e. 65/lakh/year; and reduce estimated mortality due to TB (per 100,000) i.e. 3 and to achieve zero catastrophic cost for affected families due to TB. There are other outcome indicator details present in the document. The implementation of NSP will require lot of resources – human as well as financial so as to achieve dream of TB elimination by 2025.

Malnutrition and tuberculosis are both problems of immense importance in underdeveloped regions of the world. These two problems tend to interact with each other. Under nutrition is highly prevalent among people with tuberculosis. This is also a risk factor for progression from tuberculosis infection to active tuberculosis disease. The government is planning to provide nutritional support to those undergoing TB treatment. The move aims to help speed up treatment and provide faster recovery from TB.[3] Nutritional support will also help to cover wage loss for patients and support them to complete treatment. Efforts to provide nutritional support have been attempted at many places with improvement in treatment outcomes. NSP envisages providing nutritional support to all cases put on treatment in uniform bond.

WHO's recently announced Global Plan to Stop TB highlights the need to expand DOTS through ‘‘standardized treatment, under proper case management conditions, including directly observed treatment to reduce the risk of acquiring drug resistance and support of patients to increase adherence to treatment and chance of cure’’.

However, the value of the direct observation component of DOTS has been questioned in a recent systematic review, in which it was suggested that direct observation of treatment is unnecessary and disrespectful of patients. Both self-administered treatment and treatment observation by a family member have been proposed as acceptable alternatives. A new concept by the name of 99 DOTS is also being adopted by the programme to strengthen adherence.[4] Under this component, patients send a free call each time when they take their medication, so that providers can monitor adherence records. The calls are toll-free, so patients do not have any additional costs.

As per the past experience, high cure rates can be achieved via direct observation of treatment given by a person, accountable to the health system and accessible to the patient. The primary responsibility of a TB control programme to patients and to the community is to ensure cure while preventing drug resistance. Direct observation of treatment is the only current documented means to meet this commitment.[5] A study that focused on adherence to DOTS, carried out in India, and verified the need to focus research on addressing the disease from the perspective of patients and health professionals, who are the essential elements in this process.

With the growing menace of MDR-TB, this is a dire need of introduction of newer effective drugs to make regimen more effective and of less duration. Recently introduced Bedaquiline and Delamide have been found to be promising.

There are various co-morbidities/medical conditions which are risk factors for TB[6] and for poor TB treatment results, while TB can complicate the disease course of some diseases. People living with HIV are more likely to develop TB than persons without HIV. TB is the most common presenting illness among people living with HIV, including among those taking ART and it is the major cause of HIV-related death. HIV has also been a known cause of amplification of drug resistance. Managing both the conditions simultaneously is pre-requisite to control menace of TB. Among diabetics TB rates are higher than in the general population, and diabetes is common co morbidity in people with TB. Diabetes can worsen the clinical course of TB, and TB can worsen glycaemic control in people with diabetes.

Tobacco smoking increases the risk of TB 2–3-folds, and is associated with poor TB treatment results. Efforts to reduce smoking in TB cases help in getting better treatment outcomes. Similarly harmful use of alcohol increases the risk of TB 3-folds and is also a strong risk factor for poor TB treatment adherence. In countries with high prevalence of alcohol use disorders, in intermediate and low-incidence countries where TB has become highly concentrated to certain vulnerable groups, harmful alcohol use can be an important population level risk factor for TB.

Importance of psychosocial support in cases of MDR patients has been highlighted prominently across various forums. Time to time support from the team would go a long way in treating the patients. Various studies state that psychosocial support is a crucial component of treatment for MDR-TB in order to ensure completion of complicated treatment regimens and enable psychosocial rehabilitation after treatment. This component needs to be addressed in our TB control programme, if we are thinking of eliminating TB by 2025. Need for follow up after treatment has also been high lightened. As per *Standards for TB care in India*, Standard 8 states that after completion of treatment the patients should be followed up with clinical and/or sputum examination at the end of 6 and 12 months to rule out any relapse.

There are a number of issues related to rehabilitation which should be addressed after patient has completed treatment.[7] Some of them have side effects (especially DRTB drugs), nutritional support that receives little attention in national programs, constant stigma and discrimination that individuals face from friends, family and even healthcare workers and returning to jobs or to their education is a challenge as many workplaces and educational institutions do not provide for extended periods of leave or absence. This is costly both in terms of time and money. In families where the primary "bread winner" needs to undergo intensive treatment, the reduced income can have a devastating effect. Studies suggest that post-treatment rehabilitation systems needs to be put in place and are designed based on the needs. Low cost interventions like peer-to-peer counselling should be adopted.

Nowadays, studies have shown that the TB precision treatment including therapeutic drug monitoring (TDM) may help eliminate TB. It has been shown that in combination with clinical and bacteriological data, TDM can be a decision making tool for clinicians to successfully treat even the most complicated TB cases such as MDR/XDR, drug–drug interactions in patients with HIV, etc.[8]

For fast and timely elimination of TB from India, RNTCP needs to be more strengthened not only in terms of financial stability but also its more involvement with the civil society. Moreover, TB advocacy needs to be more active which help to gear with stigma and other barriers that patients encounter. To conclude, the dream of TB elimination by 2025 cannot be achieved by only DOTS, it requires a lot beyond DOTS, although the dream is not unachievable.

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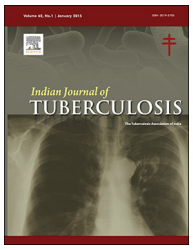

Review Article

Extensively Drug-resistant Tuberculosis (XDR-TB): A daunting challenge to the current *End TB Strategy* and policy recommendations

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ABSTRACT

Extensively Drug-resistant Tuberculosis (XDR-TB) has emerged as one of the most formidable challenges to the *End TB Strategy* that has targeted a 95% reduction in TB deaths and 90% reduction in cases by 2035. Globally, there were an estimated 55,100 new XDR-TB cases in 2015 in 117 countries. However, only one in 30 XDR-TB cases had been reported so far. Drug susceptibility test (DST) is the mainstay for diagnosing XDR-TB, but the lack of laboratory facilities in the resource-limited endemic countries limit its uses. A few new drugs including bedaquiline and delamanid, have the potential to improve the efficiency of XDR-TB treatment, but the drugs have been included in 39 countries only. The costs of XDR-TB treatment are several folds higher than that of the MDR-TB. Despite the financing from the donors, there is an urgent need to fill the current funding gap of US$ 2 billion to ensure effective treatment and robust surveillance. In the review article we have addressed current update on XDR-TB, including surveillance, diagnosis and the interventions needed to treat and limit its spread, emphasis on extensive financial support for implementing of current recommendations to meet the goals of *End TB Strategy*.

1. Introduction

In 2006, the World Health Organization (WHO) and the US Centers for Disease Control and Prevention first introduced the term Extensively Drug-Resistant Tuberculosis (XDR-TB).[1] XDR-TB is defined as TB that is caused by strains of *Mycobacterium tuberculosis* which are resistant to isoniazid and rifampicin, one of the fluoroquinolones (such as, moxifloxacin or ofloxacin) and at least one of the second-line anti-TB injectable aminoglycosides (such as, amikacin, capreomycin or kanamycin).[1,2] Before May 2016, drug-resistant TB was mainly focused on multi-drug resistant (MDR) TB (resistant to both isoniazid and rifampicin). But since then, WHO mentioned that TB resistant to rifampicin should be treated as MDR-TB irrespective of the resistance to other TB drugs.[3] *M. tuberculosis* has a great ability to become resistant to anti-tubercular drugs.[1] Drug-resistant tuberculosis can develop due to inadequate or inappropriate drug therapy in patients with tuberculosis (acquired resistance) or by direct infection with drug-resistant strains of tubercle bacilli.[2,4] However, studies have proven the stepwise evolution of drug resistance even after appropriate adherence to the Directly Observed Therapy Short-course (DOTS) strategy in HIV-positive TB patients.[5,6] In fact, the

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evidence demonstrates that implementing the DOTS strategy without doing drug susceptibility testing (DST) during diagnosis might be responsible for the evolution of XDR-TB.[6]

A documented case study in Norway showed, how a Multi-Drug Resistant TB (MDR-TB) case turned into an XDR-TB case even after successful DOTS treatment at a tertiary hospital. Furthermore, isolation of nine genotypes of *M. tuberculosis* from the same patient reflects the rapidly evolving ability of the tubercle bacilli to acquire resistance mutations multiple times within a short period.[7]

In 2006, WHO developed the *Stop TB Strategy* based on the success of DOTS and the strategy pays significant attention to drug resistance surveillance.[3] Despite considerable progress in the fight against TB, XDR-TB has emerged as a formidable challenge to the ongoing efforts. In 2015, WHO adopted an ambitious *End TB Strategy* by targeting a 95% reduction in TB deaths and 90% reduction in cases by 2035.[3,8] To achieve the new goals, an all-out effort is paramount to address surveillance, treatment, resource allocation and capacity building concerning XDR-TB. TB elimination by 2050 is nearly impossible if the incidence of TB continues declining at the current rate of 1.5%/year.[9] In this paper, we address the existing challenges to the global control strategy of XDR-TB, new opportunities and offer some relevant recommendations.

2. Surveillance

The *Stop TB Strategy* emphasized a comprehensive and efficient national surveillance system for the best estimation of TB cases.[10] However, only 21 of the 40 high burden countries have surveillance data on second-line drug sensitivity test (DST).[3] It is worth noting that XDR-TB represents a significant problem not only in high-burden countries but also worldwide. In November 2007, WHO reported 41 countries as having at least one case of XDR-TB.[11] By the end of 2015, the number of such countries increased to 117.[2] An estimated 9.5% of MDR-TB cases had XDR-TB.[3] WHO estimated that there were 480,000 new cases of MDR-TB worldwide in 2015 with an additional 100,000 patients of rifampicin-resistant TB (RR-TB). Only three countries (India, China, and the Russian Federation) were accounted for 45% of those 580,000 cases who were eligible for the treatment of MDR-TB.[3] Following WHO's assessment, there were at least 55,100 new XDR-TB cases in 2015. However, only 3.4% of those cases were reported.[3] Therefore, the vast number of undiagnosed cases pose a serious threat, particularly in the highly endemic developing countries. The Strategic and Technical Advisory Group for TB of the WHO identified 30 countries with high TB burden and another 30 countries with high burdens of MDR-TB (totally 40 countries due to overlapping) and 14 of them are also included in high-TB and TB with HIV burden groups.[3] Among them three countries (Angola, Congo, and Liberia) have never done any drug resistance survey, one country relies on data collected before the year 2000 (Sierra Leone) and five countries (Brazil, Russian Federation, Central Africa Republic, DPR of Korea and Papua New Guinea) have subnational data only.[3]

Surveillance of pediatric XDR-TB cases is also grossly inadequate.[12] In 2015, 6.3% of the new TB cases were less than 15 years old children. The large-scale deficiency in TB surveillance has a substantial implication for national and global TB policies as children comprise between 20 and 40% of the total TB cases in high TB burden countries.[12,13] Estimates from 2013 show that about 600,000 children worldwide yearly needed evaluation as household contacts of MDR-TB and approximately 30,000–50,000 required active treatment for it.[14]

People with HIV-positive status were accounted for 1.2 million TB cases in 2015 (11% of all new cases).[3] The proportion of those cases were the highest in the Africa Region (31%) of WHO.[3] HIV-positive people have an almost 30 times increased chance of developing TB than those with HIV-negative status but only 55% of the reported TB patients had been done HIV tests in 2015.[3] High endemicity of HIV-infection and MDR-TB, lack of drug resistance surveillance and poor health services infrastructure resulted in high rates of mortality and an expanding epidemic of XDR-TB in sub-Saharan Africa.[5,6,15,16] If antiretroviral therapy (ART) is introduced within the first eight weeks of starting the anti-tubercular drugs, the survival of patients with concomitant XDR-TB and HIV/AIDS significantly improves.[15,17]

In 2015, more than 6 million new TB cases were reported, mainly due to a substantial increase (34%) in notifications in India.[3] Still, globally 4.3 million incident cases were not notified.[3] In the same year 132,120 MDR/RR-TB cases were reported that was a substantial 20% increase in four (China, Nigeria, Ukraine, and the Philippines) of the high burden countries from the previous year.[3] If all of the notified TB cases had been undergone DST, an estimated 580,000 MDR/RRTB,[3] and 55,100 XDR-TB cases would have been diagnosed in that single year, and more than half of those would have come from India, China, and the Russian Federation. This huge detection gap is essentially due to under-reporting of the diagnosed TB cases (like in the private health sector) and under-diagnosis due to inadequately equipped diagnostic facilities and lack of training to the health care team.[3,13]

3. Diagnosis

The clinical signs and symptoms of XDR-TB are similar to those of drug-susceptible tuberculosis.[2,18] Therefore, DST of the identified bacilli is necessary for the diagnosis of XDR-TB.[2,3,19] However, only 60% of pulmonary TB patients had a bacteriologically confirmed (smear or culture positive or confirmed by a rapid diagnostic test such as, Xpert® MTB/RIF) diagnosis and rest were being diagnosed by non-standardized diagnostic methods such as clinical symptoms and/or chest x-ray in 2014.[2,20] In 2015, only one-third of the 3.4 million notified new bacteriologically positive, and half of the previously treated TB cases were reported to have had DST for rifampicin.[3]

DST is the mainstay of diagnosing XDR-TB, but its use is widely limited because of the lack of laboratory facilities in the resource-limited endemic countries. In 1994, the WHO/Global Laboratory Initiative established the *Supranational Reference Laboratory Network* (SRLN) to support global TB drug resistance surveillance.[11] Initially, 14 laboratories volunteered to join the SRLN to support the project, and most of those laboratories were in Europe.[20,21] The SRLN has permanent TB laboratories to provide accurate culture and DST. Each of those laboratories

supports at least two countries for DST. They also offer External Quality Assessment for drug resistance surveys and provide training on DST. By the end of 2015, the SRLN expanded to 36 laboratories supporting nearly 156 *National Reference Laboratories* (NRLs) worldwide.[3,20] But, there are only two of those laboratory facilities in Sub-Saharan Africa.[6] Moreover, inadequate staffing, excessive workloads, and scarce laboratory infrastructure are some drawbacks of current NRLs.[22] Due to the lack of laboratory infrastructure in most of the developing countries, only 24% of the new bacteriologically confirmed (3.4 million) and 53% of previously treated TB patients underwent DST by the end of 2015.[3] Globally, 132,120 cases of MDR/RR-TB cases were reported to be diagnosed in the same with vast disparities of coverage between countries.[3]

In 2010, WHO recommended the use of Xpert® MTB/RIF as a primary diagnostic test in all suspected cases of pulmonary TB. Consequently, as of the 31st December 2014, 116 countries (all of the high MDR-TB burden countries) have included the Xpert® MTB/RIF as the initial diagnostic test for diagnosing drug-resistant TB.[2] By the end of 2015, only 15 high burden countries (accounting for 10% of all the incidence cases) had adopted it as the primary test for any patient with the signs and symptoms of TB. In 2016, WHO has recommended a DNA-based test (known as MTBDRsl) that identifies genetic mutations in MDR-TB strains, making them resistant to fluoroquinolones and injectable second-line TB drugs. This test yields results in just 24–48 h and recommended the diagnostic test for use in NRLs.[23] This test is a critical prerequisite for identifying MDR-TB patients who are eligible for the newly recommended regimen and eventually can prevent the development of XDR-TB.

4. Treatment

Globally, in 2015, 7234 XDR-TB patients were enrolled on treatment in 58 countries and territories. Most of those cases were from India (2130), South Africa (719), the Russian Federation (1205) and Ukraine (1206).[3] Treatment outcomes of XDR-TB vary widely depending on drug regimens, duration of treatment, the prevalence of TB and HIV and even on geographical location.[17,24] Usually, the outcome correlates with the spectrum of drug resistance.[25] About 250,000 deaths were reported from MDR/RR-TB in 2015. The latest data from cohort studies show a treatment success rate of 83%, 52% and 28% for TB, MDR-TB, and XDR-TB respectively.[3] Previously a meta-analysis of some observational studies found an overall 44% success rate for XDR-TB treatment.[25] In high-burden countries, this rate may be even lower. In South Africa, less than 20% of XDR-TB patients became culture-negative after the treatment, and it was not dependent on HIV status.[17] Another study in South Africa has demonstrated that younger patients had better treatment outcomes whereas, the presence of comorbidities, such as chronic obstructive pulmonary disease and hypertension was associated with poorer outcomes.[26]

There are a few new drugs under development, and they have the potential to improve our capacity to treat XDR-TB.[27,28] For example, bedaquiline (diarylquinoline compound), delamanid (nitroimidazole), have shown rapid culture conversion.[25,27,28] However, these drugs were not available before April 2015 to the NTP in most of the countries where XDR-TB is most prevalent.[27] Since then The *Bedaquiline Donation Program* has been supplying bedaquiline to the eligible low and middle-income countries at an affordable price.[8] The donation has been provided through USAIDS's agreement with the *Global Drug Facility (GDF)* of the *Stop TB Partnership* to increase the access of the high burden countries to quality-assured medicine.[3,8] As a result, by the end of 2015, Bedaquiline and delamanid had been included in the drug regimens for treating MDR-TB and XDR-TB in 70 and 39 countries respectively.[3,28]

Very recently (in 2016), the WHO has published treatment guidelines (updated version of 2011 recommendation) for drug-resistant TB, primarily focusing on the composition of treatment regimes, effectiveness and safety of shorter regime, and roles of surgery.[28] The recommendation for a conventional drug regimen suggests at least five effective TB medicines during the intensive phase, including pyrazinamide and four core second-line TB medicines; (a) one chosen from group A (Levofloxacin, Moxifloxacin, Gatifloxacin), (b) one from group B (Amikacin, Capreomycin, Kanamycin), and (c) at least two from group C (Ethionamide (or Prothionamide), Cycloserine (or Terizidone), Linezolid, Clofazimine). If the minimum of effective TB medicines cannot be composed as above, an agent from group D2 (Bedaquiline, Delamanid) and other agents from D3 (p-aminosalicylic acid, Imipenem-cilastatin, Meropenem, Amoxicillin-clavulanate, Thioacetazone) may be added.[28]

The treatment success rates with the conventional drug regimens are not very high. Also, many drugs are costly with significant adverse effects and are given for a long duration. So, WHO recommends a shorter drug regimen of 9–12 months instead of the conventional regimen in RR-TB or MDR-TB patients who have not been treated with any second-line drugs before and who do not have or have not been considered to have any resistance to fluoroquinolones or the second-line injectable agents.[28] At least 23 countries of Asia and Africa have reported the use of shorter treatment regimens for MDR-TB and RR-TB with a success rate of 87–90%.[3]

In RR-TB patients, a regimen containing at least five TB drugs is recommended by the WHO during the intensive phase (first two months). Pyrazinamide and four other second-line drugs – one each from group A and B, and two from group C should be used. If the patient is resistant to any of those drugs, an agent from group D2 or D3 can be included in the regimen. High-dose isoniazid and ethambutol can be added to reinforce the treatment process. WHO also recommends that any RR-TB patient – who does not have isoniazid resistance or if it is unknown, should be treated with a recommended shorter or a conventional MDR-TB regimen that includes isoniazid.[28]

Surgery is an old approach to TB treatment that has gained new importance in the WHO's recommendation.[28] Studies have shown that cavities contain highly concentrated organisms, and they are the sites for the development of drug resistance.[29] Surgical excision (lobectomy or wedge resection) will substantially reduce the resistant organism burden and increase the rate of treatment success.[29] WHO mentions that an elective partial lung resection might be used in addition to the recommended drug regimen in RR-TB and MDR-TB cases.[28] A phase 1 study is under way in Belarus with XDR-TB patients

using autologous bone marrow-derived mesenchymal stem cell transfusions to reinvigorate pulmonary parenchymal immune responses to enhance mycobacterial clearance.[30] There is a recent technology, known as *iChip*, a new in situ cultivation method, aiming to increase access to a greater diversity of microorganisms.[31] The *iChip* has already developed a novel antibiotic *Teixobactin*, with significant potency against Gram-positive bacteria and mycobacteria and it appears effective against the development of resistant organisms.[31]

Despite all the new diagnostic tools and new drugs, MDR-TB diagnosis and treatment are still in major crisis. In 2015, only 125,000 cases (20% of the estimated eligible people), were included for the treatment of MDR-TB and 10% of the MDR/RR-TB incidence cases were successfully treated.[3] Five countries (India, China, the Russian Federation, Indonesia, and Nigeria) had more than 60% of the burden of that gap between diagnosed and treated cases of MDR/RR-TB. In 2013, on an average, the success rate of MDR-TB treatment was 52% globally. A total of 910,000 HIV-positive patients (78%) were started on antiretroviral therapy (ART) in 2015. But TB preventive treatment in those vulnerable population should be expanded.[3]

5. Cost of treatment in the contexts of economic and socio-political changes

The costs of XDR-TB and MDR-TB treatment are several folds higher than that of the drug-susceptible TB. Data shows that the cost of treatment usually ranges from $100 to 1000 and $2000 to 20,000 per patient for drug-susceptible TB and MDR-TB respectively. But those costs differ among the countries according to the income-groups they are in.[3] In 2014, each drug-susceptible TB patient needed on an average US$14,659, US$840, US$273 and US$258 in high-income, upper middle-income, lower middle-income, and low-income countries respectively. The average costs for treating each MDR-TB case were US$83,365, US$5284, US$6313 and US$1218 respectively.[32] In most of the high MDR-TB burden countries, the treatment cost for only MDR-TB is more than their individual per capita gross national income.[3] In 2015, the direct treatment cost for a drug-susceptible TB and an MDR-TB in India was US$215 and USD$ 7500 respectively.[33] In the same year, in the USA, the average direct cost to treat drug-susceptible TB was US$18,000 whereas, the average treatment cost of a patient with XDR-TB was $494,000.[2] When productivity losses such as loss of income were included, those costs were even higher.[2]

The estimated funding required for the management of TB in middle and low-income countries in 2016 was about US$ 8.3 billion per year (excluding research and development) that would increase to US$12.3 in 2020.[3,8] But just over US$ 6.6 billion was available for them to spend, of which domestic sources funded 84%. Nevertheless, the NTPs of those countries still depend on the financing (nearly 90%) from international donors (mostly from Global Fund).[8,34,35] WHO data showed that those countries were US$2 billion short of the money they needed and half of the deficiency was related to spending for WHO's Africa region.[8,36] This gap is estimated to be US$6 billion in 2020 if the current trend of funding continues.[3] Treatment for MDR-TB faced the biggest challenge regarding the funding gap. Though MDR-TB needs several times more money than drug-susceptible TB to treat, only US$1.7 billion was funded for it in 2016 (whereas, drug-susceptible TB got US $6.4 billion funding).[3,8] Based on the estimates by WHO, that amount should be doubled in the following five years.[8] If full funding were available by 2016, more than 90% MDR-TB patients in seven high MDR-TB burden countries (India, Indonesia, South Africa, Ukraine, Pakistan, Philippines, and Kazakhstan) would have been detected and treated.[36] It is interesting to note that there was no direct funding for the treatment of XDR-TB.[34,36]

The ongoing global economic recession has made the funding situation more challenging. From 1991 to 2007 (before the recession) the European Union (EU) had nearly 85% case detection rates. Subsequently, those rates declined by 5.22% during the recession (2008–2011). The mass migration of refugees to the EU has increased the further risk of increasing the number of TB cases, particularly XDR-TB in the continent. There is a possibility of underreporting being attributable to that population movement. Unfortunately, the data on TB trends in refugees to the EU are not complete as there is a lack of accurate denominators for the migrants' native countries.[37]

Tuberculosis control has been an easy target for spending cuts. Some governments (e.g. Ireland, Greece, Latvia) substantially reduced their spending on communicable disease control and public health services.[21] If the case detection rate declines, diagnosis of the XDR-TB cases will decrease, and subsequently, the treatments will be affected. According to The European Centre for Disease Prevention and Control, active and untreated TB patients may infect 10–15 people per year.[21] Unfortunately, short-term budgetary gains by lowering cost in case detection can lead to an increase in long-term treatment costs. For example, In the USA, tuberculosis spending was reduced due to the fiscal crisis in the 1970s. Initially, US$ 100 million was saved, but an outbreak of MDR-TB and XDR-TB subsequently cost more than US$ 1 billion.[37]

6. Recommendations

Control of XDR-TB is one of the most formidable tasks for the success of current *End TB Strategy*. XDR-TB is gradually gaining importance in the current global initiative to manage drug-resistant TB. However, there are numerous challenges (mentioned earlier) for effective control of this emerging public health threat. We recommend the following strategies for effective control of XDR-TB.

6.1. *Improvement of quality of care and resources allocation*

The mortality rate of people dying from TB (case fatality rate or CFR) should be decreased from 17% (in 2015) to 10% in addition to the declining of TB incidence to achieve the 35% reduction in TB deaths within 2020. The CFR of TB in 2015 ranged from below 5% to more than 20% globally, revealing the significant inequalities among countries in access to the diagnosis and treatment of all forms of TB that should be addressed.

At least nine countries (Afghanistan, Burkina Faso, Congo, Chad, Papua New Guinea, Somalia, Sierra Leone, South Sudan

and Yemen) that notified more than 5000 pulmonary TB cases in 2015 had no capacity to do DST. So, there has been a felt-need for a rapid scale-up of the laboratory capacity with increased deployment of new technologies globally, so that every person presenting with the signs and symptoms of TB could be undergone first-line DST.[2,11] Studies also show that the first preventive measure to reduce the burden of XDR-TB is to provide efficient treatment of patients with MDR-TB.[3] WHO predicts that, without developing new drugs or vaccines against TB, it will not be possible to meet the 2035 TB elimination goal.[38] Hence, there is an urgent need to expedite the current progress in the global scaling up of MDR-TB services and care, including expansion of diagnostics and improving the capacity to treat patients. Some of the countries have started implementing the 'Test and Treat' strategy as the standard of TB care, where DST is done routinely in every TB patient to initiate the appropriate treatment regimen from the very beginning. In Mumbai (India) the number of MDR-TB cases had increased by eight-fold from 2011 to 2013 when they made the Xpert® MTB/RIF technology accessible to the TB centers.[9,39]

WHO recommended a short-term regimen of TB drugs have shown excellent success rate throughout the world. It has a short duration, low cost (about $800 per course if in GDF donation program enrolled) and less possibility of adverse effects from drugs than the conventional regimen. So, it should be included in the NTPs of all the countries especially the high burden countries.

Global partnerships and financing efforts among national, international funding agencies and professional organizations have contributed to substantial progress in strengthening laboratory services and improving testing capacity and using new and effective drugs. Due to the awareness of governments and funding agencies, new rapid and accurate diagnostic technologies like Xpert® MTB/RIF, liquid culture, and the line probe assay have been introduced in the national laboratories to be integrated into the national TB programs and new drugs can be used with low cost due to drug donation programs (like Bedaquiline donation program). As a result, patients are diagnosed early and been treated effectively with fewer complications. The increasing use of these new diagnostic tools has contributed to significant progress in the detection of XDR-TB and MDR-TB cases and the use of WHO-recommended regimens has shown in increased treatment success rates.

Although the initiation of the Xpert® MTB/RIF for diagnosis in decentralized settings is an important step forward, the roll-out of it has opened up some critical gaps in implementation. The deployment of this technology has been constrained by its costs and the infrastructure requirements in several endemic regions. These factors have made scale-up and decentralization is challenging in many endemic countries, thus resulting in an uneven implementation and decreased impact on XDR-TB and MDR-TB populations. It has been evident that only the introduction of new and efficient technologies alone does not result in improved patient management on TB epidemics. For new technologies to be most effective, there is a need for comprehensive solutions tailored to the specific settings are also required.

Raising public awareness about TB by using different mass media and social media is also crucial for its management. Regular communication with some targeted and high-risk groups should be continued. Advocacy in a responsible manner to different national and international donors is also essential.[9]

No strategic plan will be successful without the high-level political commitment. It is necessary to make sure that National TB Control Program is adequately funded and utilize the resources in innovative ways. As most of the high TB burden countries have very limited resources, the use of expensive modern diagnostic tools by pooled testing strategy can significantly increase the affordability.[40] Strict regulatory policies should be implemented by the national decision makers, like, India has made several bold policies and plans like imposing ban on the serological tests and implementing mandatory notification of all TB cases. Even giving more autonomy to the local policy makers can make a major contribution to the war against TB, for instance, Mumbai City (India) authority has developed its TB control plans working with other national and international partners in order to get technical and monetary supports and involving the local communities.[9,39] The engagement of the private sector in TB care is crucial in two ways. Firstly, in some of the high TB burden and high MDR-TB burden countries, a large proportion of patients seek medical help from the private sector. Secondly, by educating private service providers and providing incentives to the service delivery centers especially for delivering service to specific patients (like XDR-TB cases, HIV/AIDS patients with TB) will improve case notification rates and ensure the quality of care.[2]

Ongoing economic stagnation has created uncertainties regarding the flow of financial support from the traditional donor countries.[36,41] Therefore, it is vital to look for alternative sources, like the emerging economies. The BRICS (Brazil, Russian Federation, India, China, and South Africa) health ministers have already identified TB as one of the priority areas for future cooperation.[22]

Governments of the high burden countries are also required to increase the expenditures on health care. In 2014, most of the countries spent less than the WHO recommended 6% or more of the gross domestic product (GDP) in this sector. So, out-of-pocket costs were more than 45% of the total health expenditures in 11 of the 30 high TB burden countries.

6.2. *More research*

At least $2 billion per year is estimated to be required for TB research, which has never been achieved in last decade (never exceeded $0.7 billion per year). However, for the development of new diagnostic tools and drugs, global donors should ensure the availability of this funding.[3,36]

WHO recommended four diagnostic tests in 2016 for early and accurate diagnosis of TB and the detection of resistance of tubercle bacilli to the first and second-line drugs. A new diagnostic tool called *GeneXpert Omni* and a next generation cartridge named *Xpert Ultra* are under review of WHO that would further strengthen the diagnosis of XDR-TB.[3]

Development of new, effective and affordable drugs is the key to the control XDR-TB. The development of resistance by any pathogenic bacterium is a natural phenomenon. There is a

risk of "running out" of new antibiotics, developed by traditional technology, like screening extracts of microorganisms for antimicrobial activity. However, *iChip* technology can certainly develop a new generation of robust anti-tubercular drugs if appropriate steps are taken by the stakeholders of the *End TB Strategy*.[31,42,43] Several new drugs (e.g. delamanid, bedaquiline) have emerged, but their adoption has been varied in different countries. If these products are not validated by national organizations (e.g. National Tuberculosis Institute) and policies are not implemented to incorporate them in national drug strategies, the benefits of new technologies and drugs are unlikely to reach the target populations.[9]

International experts have suggested the screening of latent TB infection (LTBI) aiming to benefit from preventive therapy or follow-up with careful clinical observation, thus reducing the future development of TB disease (and also MDR-TB/XDR-TB).[21] Preventive therapy for MDR-TB contacts (particularly for children) is proven to be effective to stop developing the disease.[44] However, further research needed (particularly in high-burden countries) to generate evidence on; (a) multi-center randomized control studies, especially involving the new drugs and regimens, (b) adverse events, pharmacokinetic studies of different medicines, and safety (especially in pregnancy) (c) factors determining the optimal duration of treatment, minimum number of drugs and doses, (d) improved diagnostics and drug-susceptibility testing methods, (e) benefits and harms of chemoprophylaxis for child and adult contacts and LTBI, (f) shorter regimens, and (g) cost effectiveness and health-related quality of life. Since surgical intervention is a new addition to intervention; it is important to conduct research for better definition of the role of surgery (decisions about when to operate and the type of surgical intervention), the effectiveness of surgery, and cost effectiveness.

Another cost-effective way to control the XDR-TB would be a potent vaccine against TB. But the only licensed vaccine is the 90 years old Bacille Calmette Guerin (BCG) that provides partial protection against TB in children and unreliable protection against adult cases.[45] WHO recommends that BCG vaccination should be incorporated into national immunization programs considering the TB epidemiology of the specific country.[3] A better vaccine that is effective for a long period and that would prevent all forms of TB in all ages is needed urgently with a possibility of a booster dose in the later part of life. Also, an immunotherapeutic vaccine is necessary for individuals with active TB in addition to the drug therapy, to reduce recurrence rates and to control the incidence of drug-resistant TB. Considerable efforts are going on, and thirteen vaccines are already in different stages of development.[3]

6.3. *Intellectual property*

It is worth mentioning that ongoing disputes on intellectual property and price control by the big pharma companies can be the major challenge to making the newly promising drugs accessible to poor nations. The publicly funded research institutions of developed and developing countries along with the partnership of international organizations and donor agencies should initiate the new ventures. The *GDF* of the *Stop TB Partnership* has been supplying the TB drugs at affordable prices. For example, Bedaquiline and Cycloserine are available at a much lower price to the low-income countries.[3,4] Such global initiatives should be supplemented by the government negotiations with the generic pharmaceutical companies who can further reduce the costs of the second line drugs.

Not surprising, the medicine patent system has come in for criticism, especially by human rights agencies and advocates. WHO regards inequality and discrimination in access to anti-TB drugs the key contemporary public health challenge. Despite criticism, the Trade-Related Aspects of Intellectual Property Rights (TRIPS) did include several substantial exemptions from patent protection, exemptions that came to be known as *flexibilities*. The TRIPS flexibility allows governments to issue compulsory licenses for manufacturing and selling of patented generic drugs presumably at prices far lower than the patented versions, with remuneration paid to the patent holder. TRIPS also allows governments to engage in the parallel importation of generic medicine manufactured elsewhere. The 2001 WTO Doha Declaration stressed the availability and importance of these flexibilities for public welfare. TRIPS flexibilities have significantly increased access to medicines in India, Colombia, Indonesia, and over 50 other nations that have produced or procured generic HIV/AIDs medications. However, since TRIPS and Doha, the US Trade Representative and the pharmaceutical industry have pushed for bilateral and multilateral trade agreements, known as "TRIPS-Plus Agreements," that narrow the availability of these non-patent options.[46]

6.4. *Integrating with medical curricula*

Most of the textbooks of internal medicine and thoracic medicine have not yet included any information on XDR-TB.[4,18] A few textbooks have discussed XDR-TB, but the information is insufficient to meet the needs of new medical graduates. In most of the high-TB burden developing countries physicians do not have access to current journal articles, and they are mainly dependent on textbooks for the latest medical information. International experts and national institutions can take more proactive steps to reach out the new medical students and future practitioners worldwide. *e*Learning can provide new and updated knowledge and skills to implement the management of XDR-TB successfully. Internet-based and self-directed learning on tuberculosis and co-morbidities, digitalized health information and surveillance system can effectively address the needs of primary care practitioners.[47] In fact, in the *End TB Strategy*, WHO is scaling up the TB response in the post-2015 era through information and communication technologies.[47]

The world is making slow but tangible progress in XDR-TB management, but more work is needed to meet the goal of ending the TB epidemic. With political commitment, dedicated leadership, active cooperation between public and private sectors, and active support from donors and civil society, it is indeed possible to end the TB epidemic.

Conflicts of interest

The authors have none to declare.

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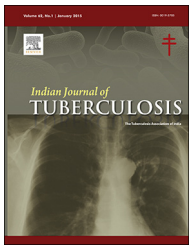

Review Article

Parotid tuberculosis

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ABSTRACT

Parotid gland tuberculosis is an uncommon manifestation of one of the most common infections even in the developing countries, caused by Mycobacterium tuberculosis. There are no specific symptoms or clinical signs of parotid tuberculosis, and such an infection most commonly presents as a slow growing painless parotid mass. Because of its rarity, tuberculosis of parotid gland is often mistaken for a malignant growth, and it most commonly gets diagnosed after superficial protidectomy. Complete cure is possible with standard antituberculous therapy. Most of our knowledge about this rare entity comes from case reports and short case series. The authors encountered three cases of parotid tuberculosis in the last 10 years. This article aims at presenting a comprehensive review of all the available literature and thus providing detailed information and an update on parotid tuberculosis and our experience of three cases.

1. Introduction

Parotid gland tuberculosis, though most common site of salivary gland tuberculosis, is an uncommon manifestation of one of the most common infections even in developing countries, caused by Mycobacterium tuberculosis. The infection of parotid with various infectious agents may be caused by a primary infection or may occur as a part of multisystem disorder, or from bacteraemia. There are no specific symptoms or clinical signs of parotid tuberculosis, which most commonly presents as a slow growing painless parotid mass. Because of its rarity, tuberculosis of parotid gland is often mistaken for a malignant growth. It is a medically curable disease, and prognosis is very good but most commonly gets diagnosed after parotidectomy. De Paoli et al.[1] in 1893 reported the first case of secondary parotid tuberculosis. Stubenrauch et al.[2] reported the first case of primary parotid tuberculosis in 1894.

The authors encountered three cases of parotid tuberculosis in the last 10 years. This article aims at presenting comprehensive review of all the available literature and thus providing detailed information and an update on parotid tuberculosis and our experience of 3 cases.

2. Methods

Electronic searches were undertaken in MEDLINE and PUBMED using the MeSH terms "parotid" in combination with

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''tuberculosis'', ''tuberculous'', ''tubercular'', ''tuberculosis in immunocompromised patients''. A total of 212 cases have been identified in various case reports and series. All resulting titles, abstract, and full text, whenever available, were read and kept for reference, and the findings were summarized.

2.1. *Epidemiological features*

In spite of being a medically curable disease with availability of highly effective drugs and vaccine, tuberculosis still continues to be major worldwide health problem. The highest burden of tuberculosis is in India (20% of the global burden).[3] Extrapulmonary tuberculosis is not uncommon, in the head and neck region, tubercular lymphadenitis is the commonest site of extrapulmonary tuberculosis and salivary gland tuberculosis is considered very rare among head and neck tuberculosis patients. In a study by Prasad et al.,[4] out of 165 patients of head and neck tuberculosis, only three patients were found to have parotid tuberculosis. Ozcan et al.[5] concluded that tuberculosis comprises 2.5% to 10% of parotid gland lesions. It has been observed that parotid tuberculosis is more frequently encountered in Asian and African countries.[6] Menon et al.[7] in his study on head and neck tuberculosis reported that out of 128 cases, salivary glands were involved in five cases, and in the majority of cases patients' ethnic origin were Asian (89%), Caucasian (10%).

Parotid tuberculosis has been found to be more common in males as compared to females in most of the reported cases and case series.[8–10] It has been observed that parotid tuberculosis is seen most frequently in adult life, median age of presentation is 45 years, youngest reported case of 3 years of age[11] while the oldest patient was 92 years old.[12] Both sides are equally affected.

2.2. *Etiopathogenesis*

Primary parotid tuberculosis is rare, secondary involvement from an adjacent organ is more common or parotid may get involved as a part of miliary tuberculosis. Patankar et al.[13] concluded that salivary glands including parotid are relatively resistant to tuberculosis, as continuous flow of saliva prevents lodging and growth of bacilli and also because saliva contains thiocyanate ions and proteolytic enzymes such as lysozymes, which impart antibacterial property. The most common salivary glands involved in primary tuberculosis are parotid glands because of the sluggish flow of the saliva in these glands, while submandibular salivary glands gets most commonly involved in systemic tuberculosis. Carmody et al.[14] also supported the fact that parotid gland gets more commonly affected in localized spread. There are certain predisposing conditions and precipitating factors causing parotid tuberculosis.[15] These include oral injury, dental infection or carious teeth near the parotid, tonsillar infection, poor oral hygiene, Stenson's duct obstruction, medical comorbidity such as diabetes mellitus, presence of chronic disease likes such as Sjogren's syndrome, immunosuppression, malnutrition, sialolithiasis, anticholinergic and antihistaminic dugs. Tuberculous parotid infections are transmitted directly from the oral cavity infection through the stenson's duct. Infection is also known to occur through haematogenous or lymphatic route. As suggested by De Paoli et al.,[1] parotid tuberculosis can also occur from the external auditory canal through the lymph stream. It has been observed that 25% of patients with parotid tuberculosis have an associated pulmonary infection, so, hematogenous spread may occur from any primary focus.[16] Though, in most of the cases, it is hard to identify the focus and the route of spread. It can be stated that the presence of three most important factors in causing parotid tuberculosis are infection or injury of the oral cavity, blocked duct and immunosuppression. Pre-existing Warthin's tumour of parotid is also a predisposing condition. Watanabe et al.[17] concluded that the presence of lymphoid components, inflammation and necrosis and behaviour of these components in a manner similar to regional lymph nodes possibly predisposes cases of warthin's tumour to tuberculosis.

2.3. *Pathology*

There are two pathological forms of parotid tuberculosis – nodular or circumscribed form and diffuse form. In a nodular form, there is an involvement of intraglandular or periglandular lymph nodes. It may take the form of a cyst or cold abscess. It is a commoner type. The site of pathological changes could be glandular tissue or interstitial tissue. The less common diffuse form consists of small and large areas of caseation or abscesses involving the entire gland parenchyma.[15] Histopathological examination reveals well-defined components of tuberculous process – numerous tubercle formation, undergoing caseation with presence of tubercle bacilli, langhans giant and epitheloid cells, surrounded by dense fibrous connective tissue infiltrated with round cells (Fig. 1a).

2.4. *Clinical presentation*

There are no specific symptoms of parotid tuberculosis and it presents most commonly in two clinical forms – acute form and chronic form.[18,19] Acute presentation is rare, wherein the patients present with painful swelling in infra-auricular region. In commoner chronic form, the patients present with gradually progressive painless swelling mimicking a parotid malignancy. As the swelling is painless and grows very slowly most of the patients present late. In most of the reported cases, the median duration of symptoms before patients sought treatment was 6 months.[20,21] Talmi et al.[22] reported a case of painless parotid mass with isolated cervical lymphadenopathy. In another report, patient presented with painless parotid mass with discharging sinus.[23] Suleiman et al.[24] reported three cases of parotid tuberculosis presenting with discharging sinus. Herrmann et al.[25] reported a case, in which the patient presented with constitutional symptoms of tuberculosis, anisocoria, and disturbed motor function of the right eye's pupil in addition to the presence of painless right parotid mass. In a study by Lee et al.,[9] 8 out of 49 patients presented with fever, and 11 patients had associated pulmonary tuberculosis. It is extremely rare to find associated constitutional symptoms and any other site of active or inactive tubercular focus in the body. In a retrospective study of head and neck tuberculosis, it was found that only 20% patients had a coexistent site of tuberculosis and 26% had constitutional

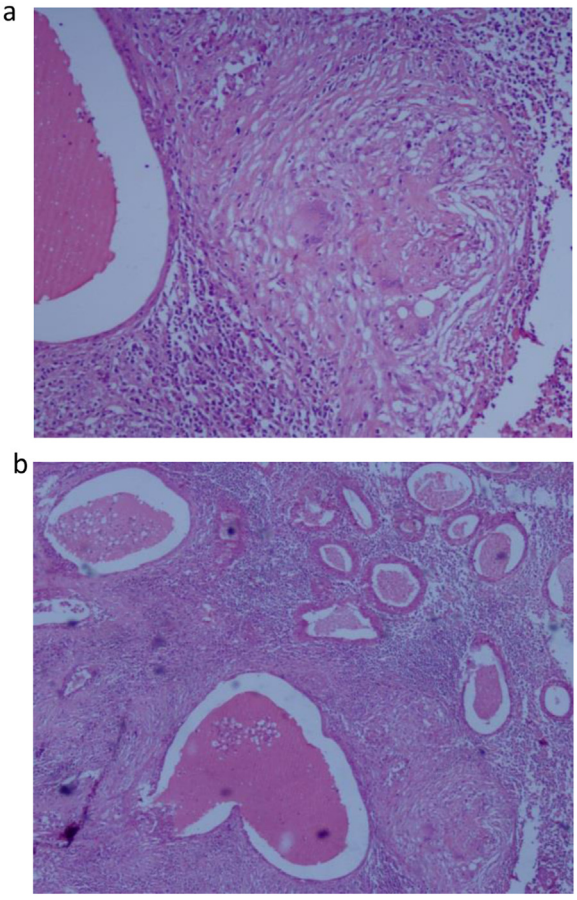

Fig. 1 – (a) Epitheloid cell granuloma and langhan's giant cells (haematoxylin and eosin ×400). (b) Warthin's tumour with epitheloid granuloma (haematoxylin and eosin ×100).

symptoms and testing for human immunodeficiency virus was positive in 65.1% cases.[7] Clinical examination reveals mobile, non-tender, firm mass in superficial lobe with no features of facial nerve involvement. Out of 3 of our patients, none of the patient presented with features suggestive of tuberculosis or any past history of tuberculosis, so, clinical diagnosis was parotid gland tumour in all the three cases, and diagnosis was confirmed after surgical procedure.

2.5. *Sequelae and complications*

Presence of abscess, fistulas, sinus and facial nerve involvement and related motor function disturbances are rare and late features.

3. Diagnosis

3.1. *Role of imaging studies*

Imaging studies have limited role in diagnosing parotid tuberculosis. In less than 50% of the patients with extrapulmonary tuberculosis, chest radiography exhibits some evidence of pulmonary disease.[26] Franzen et al.[6] in review of 20 cases of parotid tuberculosis observed that only one case had tuberculous infection on chest radiograph. Ultrasonography (US) is routinely done for parotid lesions as it is the initial study of choice to define the organ origin of pathology and its nature because of its high performance but in majority of cases US fails to diagnose tuberculous nature of the parotid pathology. US has a sensitivity of 70–80% for masses within the superficial lobe of the parotid gland.[27] US is more sensitive than computed tomography and magnetic resonance imaging for superficial lobe evaluation.[27] Thakur et al.[27] in his study observed that US shows diffuse parotid echo pattern changes with or without hypoechoic or nearly anechoic zones and with or without periparotid lymphadenopathy. Viselner et al.[28] described that gland songraphically appears increased in volume and shows a focal lesion within the parotid parenchyma with pseudo-solid, inhomogenous appearance and fairly regular margins suggesting caseous necrosis. Vyas et al.[29] concluded that parenchymal type of parotid tuberculosis can be differentiated sonographically from periparotid type. In parenchymal type, US shows a diffusely enlarged gland with heterogeneously hypoechoic parenchyma and focal hypoechoic or anechoic areas within the gland. Colour Doppler shows minimally increased colour flow around the anechoic lesions.[30] Sonographically, periparotid type appears as enlarged hypoechoic nodes in periphery of gland with parenchyma showing hyperechogenicity. Thakur et al.[27] concluded that computed tomographic (CT) scan has non-specific findings in parotid tuberculosis with an accuracy of 77–89% and shows homogenously enhancing parotid with or without contrast enhancing round areas. Various other studies have reported CT findings as a solid nodule with homogenous enhancement, multiloculated rim enhancing nodule with central lucency, contrast enhancing solid nodule with an eccentric nonenhancing microcyst. Specific CT findings reported by Bhargava et al.[31] are multiple, round, smooth thick walled rim enhancing lesion with central lucency within parotid with parenchyma showing enhancement and filling defects. Magnetic resonance imaging (MRI) alone gives 88% accuracy.[27,32] Iseri et al.[33] reported that MRI may define parotid tuberculous better than CT and US. MRI shows hypointense lesion on T1-weighted images and hyperintense on T2-weighted images with homogenous postcontrast enhancement.[27,34]

3.2. *Role of fine needle aspiration cytology (FNAC)*

Imaging studies can provide a clue for parotid tuberculosis, but definite diagnosis of tuberculosis is made only on direct histopathological examination. FNAC is indicated for all mass lesions of parotid and should always be performed as initial study to define the exact nature of the mass lesion. Iseri et al.[33] concluded that in parotid lesions, FNAC has a sensitivity of 81–100% and specificity of 94–100%. In diagnosing parotid tuberculosis, FNAC is not always contributory because of presence of necrosis in tubercular lesions. Dandabat et al.[35] and Lau et al.[36] reported that FNAC has a sensitivity of 80% and specificity of 93% when used for the diagnosis of tuberculosis. Tuli et al.[37] also supported that FNAC is of great help for

diagnosing parotid tuberculosis. But in majority of the reported cases, the diagnosis of the parotid tuberculosis was made only after excisional biopsy, as not only clinically but on FNAC also it is difficult to differentiate tuberculous from malignant lesions of parotid gland. Franzen et al.[6] observed that out of 20 cases, FNAC was diagnostic of parotid tuberculosis in only two cases. In a review of 8 cases, Kim et al.[38] observed that five cases required resection of the gland for diagnosing tuberculosis. Handa et al.[39] reported five cases of parotid tuberculosis which were diagnosed on FNAC. Rout et al.[32] reported that US-guided FNAC gives 100% accuracy. Twenty-one cases diagnosed only on FNAC and subsequently managed medically avoiding surgery, and its complications have been reported in the literature.

3.3. *Role of polymerase chain reaction (PCR)*

PCR should always be performed, when there is strong clinical suspicion and results of FNAC and imaging are inconclusive. In a review of 8 cases by Kim et al., PCR was affirmative in every case.[38]

3.4. *Role of incisional biopsy*

Incisional biopsy is contraindicated as this may result in cutaneous fistula formation which is difficult to heal.[34]

3.5. *Differential diagnosis*

Tuberculous lesions of parotid are most commonly mistaken for malignant lesions. Moreover, tubercular lesions of parotid are known to coexist with malignant lesions. Eight cases of parotid gland tuberculosis within Warthin tumour (Fig. 1b), 2 cases within pleomorphic adenoma, and 1 case within adenolymphoma have been reported in the literature. It is important to differentiate the two entities as the treatment is medical for tuberculosis while malignant lesions need surgical resection. One of our patient had double pathologies, i.e., tuberculosis with warthin's tumour.

Six cases of bilateral parotid tuberculosis have also been reported, and bilateral cases often get confused with autoimmune disorders.

3.6. *Treatment*

Since its first description in 1893 till early 20th century, the treatment of parotid tuberculosis remained operative resection.[40] But now every effort should be made to diagnose the disease before surgical resection as the treatment is medical and this disease is curative with antituberculous drugs. The medical management of parotid tuberculosis is essentially same as any other form of extrapulmonary tuberculosis.[20,38,41] Parotid tuberculosis patients respond well with standard antituberculous treatment. Treatment consists of four drug chemotherapy regimen: isoniazid (5 mg/kg BW/day), rifampicin (10 mg/kg BW/day), pyrazinamide (25 mg/kg BW/day), ethambutol (20 mg/kg BW/day) generally for 2–4 months, subsequently isoniazid and rifampicin for 6–12 months.[20,41] Multidrug-resistant organisms and drug induced liver injury require alternative regimens. CT imaging is helpful for follow-up and helpful in guiding regarding duration of therapy, complete resolution and need for any form of surgical intervention.

Since the definite diagnosis is often difficult before operative intervention, it is recommended that frozen section should be done in suspicious lesions, and where frozen section facility is not available, it is wise to do minimum resection (enucleation only) to avoid injury to the facial nerve. Facial nerve injury with its associated temporary motor disturbances has been reported. Kim et al. in a review of eight cases concluded that there was no significant difference between the surgically resected and non-resected groups in terms of treatment results or morbidity.[38]

In presence of abscess formation, effective management modality is US-guided aspiration of pus in addition to antituberculous drugs. Surgical intervention in the form of enucleation is indicated for residual enlarged parotid after effective antituberculous treatment and tuberculosis occurring within parotid neoplasm needs surgical resection. Table 1 shows conclusion of various other studies on parotid tuberculosis.

3.7. *Conclusion and recommendations*

Tuberculous cervical lymphadenitis is the most common form of extrapulmonary tuberculosis but salivary gland tuberculosis is rare and parotid gland is the most commonly involved salivary gland in tuberculosis. Primary parotid tuberculosis is rare, but secondary involvement from an adjacent organ is more common or parotid may get involved as a part of miliary tuberculosis. It is difficult to diagnose this entity only on imaging studies, US-guided FNAC helps in diagnosing this condition but of the reported cases got diagnosed only after surgery. Once diagnosed it is possible to cure pancreatic tuberculosis with standard four-drug regimen antituberculous therapy.

The authors recommend:

(a) The diagnosis of parotid tuberculosis is difficult preoperatively because of lack of specific clinical features and FNAC and imaging studies also fail to diagnose this entity. To diagnose this rare entity, it is important to keep tuberculosis of parotid as one of the differentials in patients presenting with painless parotid mass with presence of constitutional features of tuberculosis, presence of a discharging sinus, past history of tuberculosis or history of contact with tuberculosis patient, immunocompromised patient or in a patient who is from a region with a high prevalence of tuberculosis.
(b) Though FNAC commonly fails to diagnose and differentiate parotid tuberculosis from malignant lesions of parotid and incisional biopsy is contraindicated in malignant parotid lesions, in any suspicious case FNAC should be repeated preferably US-guided FNAC and the case needs to be discussed in surgical pathology board meeting as the complete cure is achievable with antituberculous drugs.
(c) Imaging studies finding and clinical suspicion and correlation also give a clue towards diagnosis.
(d) Direct histopathological examination is the best way to diagnose tuberculosis, so when FNAC and imaging studies rule out malignancy, incisional biopsy can be done.

Table 1 – Conclusion of various studies.

Author (year)	Number of cases	Conclusion
Zhonghua et al.[42] (1984)	23	–
Zheng et al.[16] (1995)	12	–
Weiner.[26] (1996)	4	Fine needle aspiration cytology followed by antituberculous therapy can avoid the need for surgery in this uncommon condition.
Franzen[6] (1997)	20	Tuberculosis is a rare cause for parotid swelling. If the right diagnosis is known before therapy, parotidectomy can be avoided.
Suleiman[24] (2001)	3	Tuberculous parotitis, particularly the diffuse form, is rare responds well to antituberculous drugs.
Handa et al.[39] (2001)	5	Diagnose on fine needle aspiration cytology and manage medically avoiding surgical intervention.
Lee et al.[9] (2005)	49	Physicians should have a high index of suspicion for tuberculous parotitis in patients with a chronic parotid lump even if the chest radiographs appear normal. Fine needle aspiration should be performed first for diagnosis and should be treated medically.
Kim et al.[38] (2005)	8	No significant differences were found between the surgically resected and non-resected groups in terms of treatment results or morbidity.
Oudidi et al.[20] (2006)	6	Symptoms of parotid gland are misleading, pathologic findings are of increasing importance for diagnosis which with the new serology techniques may improve further.
Sethi et al.[43] (2006)	3	Parotid gland tuberculosis is rare and may present in different clinical forms and patients respond well to four-drug antitubercular chemotherapy.
Menon et al.[7] (2007)	5	Isolated head and neck tuberculosis is not uncommon. Atypical presentations render diagnosis challenging, so awareness aids early diagnosis.mycobacterium cultures should be performed where possible, for diagnosis.
Oktay et al.[10] (2007)	7	Tuberculosis of the parotid gland should be considered in the differential diagnosis of patients presenting with a solitary tumour in the parotid gland.
Wei et al.[44] (2008)	4	In patients presenting with unilateral parotid nodules, tuberculosis should be considered when linearly arranged enhancing nodules are demonstrated in the superficial lobes of the glands on CT scan.

(e) In the presence of strong clinical suspicion, definite diagnosis is not possible with FNAC or imaging studies, but PCR needs to be done for diagnosis.
(f) Superficial parotidectomy should not be done; frozen is of help and when frozen section is not available, surgery should be limited to enucleation.
(g) Prognosis is good with standard antituberculous therapy.

Conflicts of interest

The authors have none to declare.

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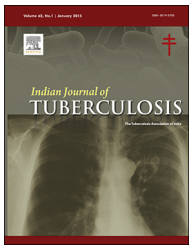

Original Article

What remains to surgeons in the management of abdominal tuberculosis? A 10 years experience in an endemic area

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ABSTRACT

Background: Tuberculosis (TB) is a common endemic disease in Tunisia. Abdominal location is rare. Early diagnosis of abdominal TB remains difficult due to its non-specific clinical presentations. The aim of our study is to highlight the characteristics of the different presentations, to characterize tools contributing to a positive preoperative diagnosis, and finally to assess the role of surgery in the management of this entity.

Materials and methods: A retrospective review from 2005 to 2015 identified 90 cases of confirmed abdominal TB managed in the Department of General Surgery of the Habib Thameur Hospital. The diagnosis was established by histopathology examination for all cases. This study was approved by the ethical committee.

Results: The mean age of the patient was 44.13 years with a sex ratio (M/F) of 0.34. We collected 56 cases of peritoneal TB, 12 cases of abdominal lymph node TB, 10 cases of intestinal TB, four cases of hepatic TB, and two cases of gallbladder's TB. For six patients, an association of many localizations was noted. The diagnosis was suspected on clinical, biological, and morphological arguments, but the confirmation was always made by surgical exploration and pathological examination of removed specimens. Surgical management was urgent in complicated cases (13.3%). Laparoscopy was performed in 71 cases (78.9%). Laparoscopic features of peritoneal TB were specific and always confirmed by histological examination.

Conclusion: Despite the wide range of examination available for the preoperative exploration of abdominal TB, diagnosis is usually late and difficult. TB is a medical condition. However, surgical exploration is frequently needed in the management.

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1. Introduction

Tuberculosis (TB) is a public health problem in many developing countries.[1] According to World Health Organization report, there are approximately 8.8 million new cases of TB each year with an annual mortality up to 1.6 million.[2] In Tunisia, TB is an endemic condition with an annual incidence of 22.4 cases per 100,000 inhabitants. Abdominal TB represents 0.6–0.8% of all forms. It is considered as one of the most common extra-pulmonary localizations of this disease.[3] The diagnosis is usually late and difficult because of the polymorphic clinical presentation and the lack of bacteriological arguments. These considerations make the surgical biopsies a crucial step in the management.[4] The purpose of this study was to provide an insight into the presentation, diagnosis, and management of abdominal TB and to assess the efficiency of surgeon's contribution.

2. Materials and methods

We retrospectively reviewed the medical records of 90 patients with confirmed abdominal TB managed in the Department of General Surgery during 10 years (2006–2015). This study excluded all the patients in whom the diagnostic confirmation was made before the surgical step. Among the 90 patients, 72 were transferred from the Gastroenterology Department. For 64 patients suffering from unexplainable ascites, the initial investigations included paracentesis for biochemical and bacterial study, colonoscopy, gastroscopy, and computed tomography (CT) for abdomen and pelvis. The other eight patients were transferred for symptomatic ileal stenosis with highly suspected obstructive Crohn's disease. Data on age, sex, medical history, clinical presentations, diagnostic procedures, and preoperative findings were recorded for all the patients. The results of the histopathological examinations of specimens were noted along with the outcome of the treated patients. The detailed data was gathered and analyzed by SPSS 20.0 software. The correlation between the laparoscopic intraoperative ''TB like'' findings and histopathological examination was studied using chi-squared and Fisher's exact test. The significance was retained for P value <0.05. The study was approved by the ethics committee of our center.

3. Results

3.1. Socio-demographic and clinical characteristics

The 90 included patients consisted in 23 males and 67 females. The sex-ratio (M/F) was 0.34. The mean patient's age was 44.13 (16–79). TB was peritoneal in 55 cases (61%) and intestinal in 20 cases (22.2%). Four cases of isolated lymph node TB (4.4%) were noted as well as two cases of gallbladder's TB (2.2%). An association between peritoneal and hepatic TB was seen in nine cases (10%). Personal or familial history of TB was noted in seven cases. The interval between onset of symptoms and diagnosis varied from 7 days to 2 years (mean is 92.3 days). Abdominal pain (66.7%), bloating and abdominal distention (60%), and weight loss (52.2%) were the most frequent symptoms. Acute intestinal obstruction syndrome was noted in 12 cases (13.3%). Ascites was the most frequent finding in the abdominal examination (71%). During a periodic medical check-up, multiple inguinal lymphadenopathies were found in one patient. Their biopsy confirmed the tubercular origin. The diagnosis of TB was incidental in one patient managed for cholelithiasis. The histopathological examination of the removed gallbladder showed TB of cystic lymph node. The patient's descriptive analysis is summarized in Fig. 1.

Descriptive analysis	
Characteristics	***n***
Age	
Mean	44.13
Range	16-79
Sex	
Male	23
Female	67
Presenting symptoms	
Abdominal pain	60
Abdominal distention	54
Weight loss	54
intestinal obstruction	8
fever of unknown origin	2
Asymptomatic	1
Abdominal examination	
Ascites	64
Hepatomegaly	2
Palpable mass	2
Inguinal lymph node	1
TB* location	
Peritoneum	55
Intestine	20
Lymph nodes	4
Gallbladder	2
Association	9

*Tuberculosis

Fig. 1 – The descriptive analysis.

3.2. Imaging features

Chest X-ray was systematically performed in the preoperative anesthetic assessment. It showed a pleural effusion in two patients with history of pulmonary TB. For the patients initially managed for terminal obstructive ileitis, the barium enema showed a ''Crohn-like'' stenosis in six cases (Fig. 2). All the patients managed for ascites of unknown origin underwent abdominal ultrasound and CT. The ultrasound was done in order to quantify the peritoneal fluid and guided a difficult paracentesis in two cases. The CT was more contributive and gave more information about the etiology in all these cases (Fig. 3).

Acute intestinal obstruction was seen in eight cases and had polymorphic appearances in the radiological examination. The CT was done in these cases to confirm the complication and to assess the severity. In one case, an ileo-ileal intussusception was suspected (Fig. 4). In another case, a pseudotumoral mesenteric mass was suspected (Fig. 5). The confirmed cases of gallbladder's TB were mistaken for calculocancer mostly for high suggestive CT's findings (Fig. 6).

The patient's radiologic examinations are summarized in Fig. 7.

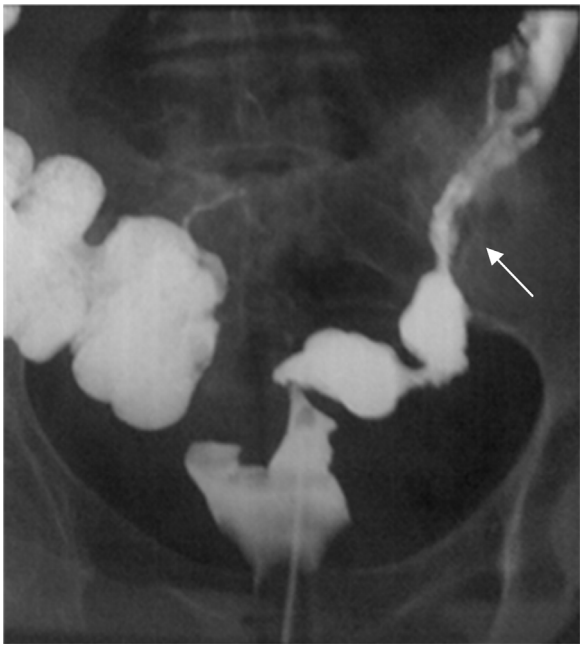

Fig. 2 – Crohn-like multiple ileal stenosis.

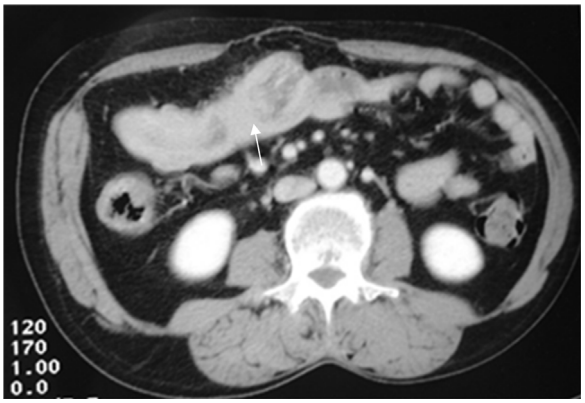

Fig. 4 – Abdominal CT's findings in a female patient with acute intestinal obstruction on a virgin abdomen: ileoileal intussusception (arrow).

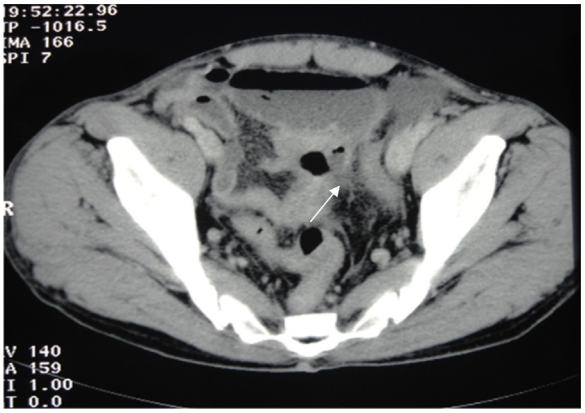

Fig. 5 – Acute intestinal obstruction caused by a mesenteric heterogeneous mass.

3.3. *Surgical procedures*

3.3.1. *The diagnostic laparoscopy*

Diagnostic laparoscopy was performed in 66 cases (73.3%). This was the final diagnostic step for 64 patients transferred for ascites with unknown origin. The procedure was urgent for two cases of suspicious acute abdomen. The laparoscopy was performed under general anesthesia in a French supine position. The access to the peritoneal cavity was made via a small umbilical incision. A trocar of 10 mm in the umbilicus and tow trocars of 5 mm in the superior quadrants were systematically inserted under vision. The exploration findings were variable. Thickened and hyperemic peritoneum was seen in all cases. Abundant ascites were seen in 61 cases (92.4%). Whitish nodules scattered over the parietal peritoneum were seen in 49 cases (74.2%) (Fig. 8).

Loculated ascites with predominant adhesions, fibrosis, peritoneal thickening were seen in three patients. Associated mesenteric adenopathies were seen in 15 cases (22.7%). For each patient, aspiration of the peritoneal liquid was done for

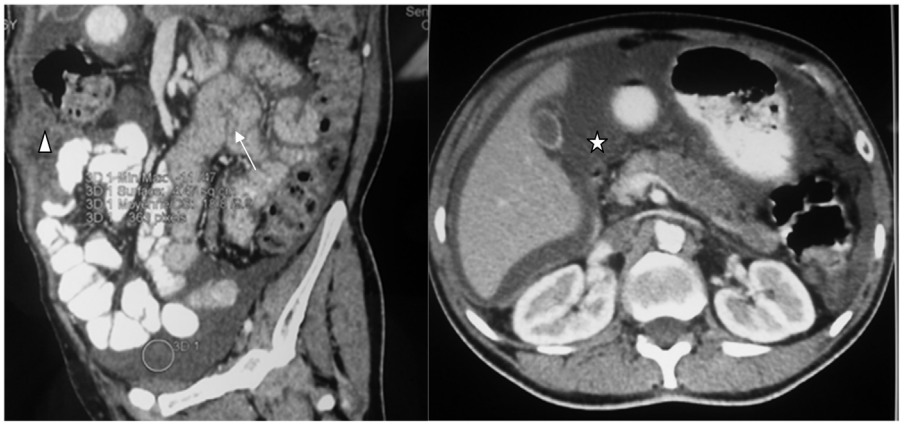

Fig. 3 – Contrast-enhanced CT showed ascites (asterisks), thickened jejunal loops (arrows), and nodular fat thickening (arrowhead) in favor of an important intraperitoneal inflammatory process.

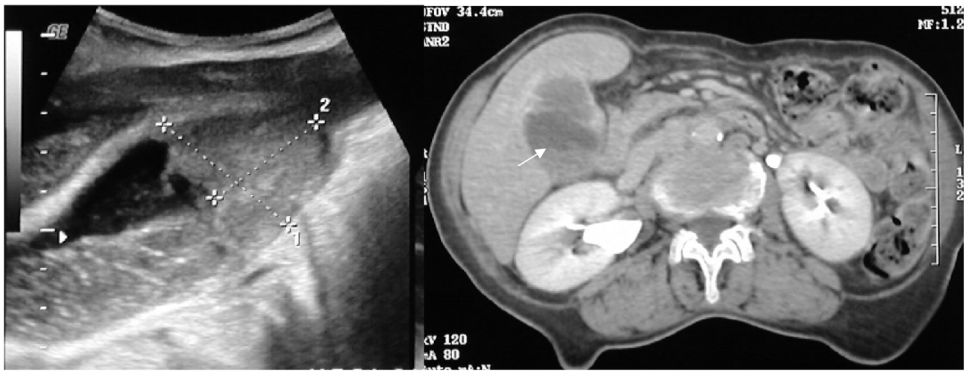

Fig. 6 – Irregular thickened wall of the gallbladder with a tissular-like mass of the fundus in favor of a carcinoma.

Radiologic examination	
examination	***n***
Chest x ray	90
Abdominal plane X ray	12
Barium enema	8
Abdominal ultrasound	69
CT abdomen and pelvis	76

Fig. 7 – Radiologic examination data.

bacterial examination as well as the biopsy of peritoneum fat and nodules.

For one patient who underwent emergent laparoscopy the exploration showed a big mesenteric mass. After conversion and biopsies the diagnosis of caseated lymph node was confirmed. In another case, multiple lymph nodes of the mesentery were found during the exploration of acute abdominal pain. The tubercular origin was confirmed after biopsies.

The hepatic biopsy was systematic in case of TB suspicion. It was positive in nine cases (10%). Diagnostic laparoscopy characteristics are summarized in Fig. 9.

3.3.2. *Intestinal resection*

Intestinal resection was made in 20 cases of intestinal obstructive syndrome. It was the case of jejunal resection in four patients, ileal resection in seven patients, and ileocecal in eight cases. End-to-end anastomosis was performed immediately. Laparoscopic approach was done in five cases (25%).

For one patient, the surgery consisted in the resection of a mesenteric mass with the sigmoid colon and 30 cm of the ileum. Colorectal anastomosis was done and protected by double ileal ostomy performed by the two remaining ileal segments.

3.3.3. *Others procedure*

In one patient, TB was diagnosed after the biopsy of inguinal multiple lymph nodes. The CT showed multiple abdomen and chest adenopathies. The patient was referred to physicians for medical treatment.

The diagnosis of TB was incidental in one patient with history of treated TB managed for cholelithiasis. The histopathology confirmed recurrent active TB of the cystic lymph node.

Two patients underwent a laparotomy for suspicious cancer of the gallbladder. A carcinologic cholecystectomy was performed. No cancerous cells were found in the specimen examination which advanced the diagnosis of TB.

3.4. *Histopathological features*

The histopathological examination confirmed the diagnosis of TB in all cases. No mycobacteriums were seen in fast acid stain of the ascites samples. The diagnosis was retained on the identification of epithelioid and giant cellular granuloma with caseous necrosis.

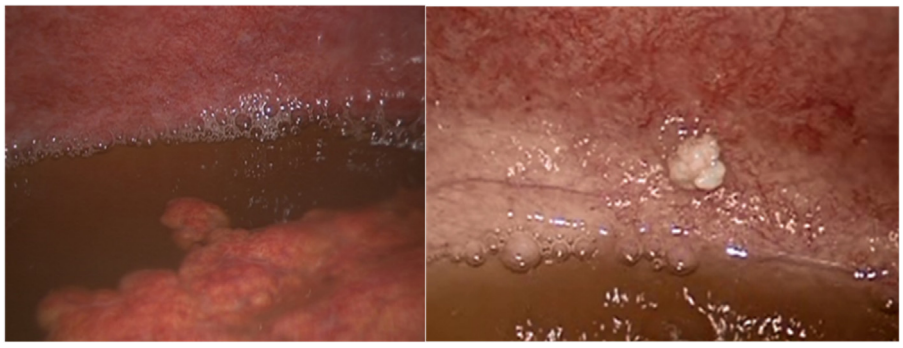

Fig. 8 – Abundant ascites with hyperemic peritoneum and parietal nodule.

Diagnostic laparoscopy	
Characteristics	***n***
Findings	
Thickened peritoneum	64
Ascites	64
.Abundant	61
.Localized	3
Whitish nodule	49
Lymph node	15
Fibrotic adhesions	3
Hepatomegaly	6
Conversion to open	1
Morbidity	
.Hemorrhage from biopsy site	3
.Subcutaneous emphysema	1
.Umbilical wound infection	8
Mortality	0

Fig. 9 – Laparoscopic exploration findings.

Laparoscopic to histopathologic correlation	
Laparoscopic findings / Confirmed TB	***p***
Thickened peritoneum	0.9
Abundant ascites	0.34
Whitish nodule	0.22
Lymph node	0.56
Fibrotic adhesions	0.11
Thickened peritoneum+ abundant ascitis	0.08
Thickened peritoneum+ Whitish nodule	0.062
Thickened peritoneum+ abundant ascitis+ Whitish nodule	0.001

Fig. 10 – Laparoscopic to histopathological findings correlation.

In order to assess the role of the diagnostic laparoscopy, the correlation between the laparoscopic and the histopathological finding was studied. The analysis was performed by chi-squared and Fisher's exact test. The significance was retained for P value <0.05. It has been proved that the association between the laparoscopic findings of ''Thickened and hyperemic parietal peritoneum'' with ''abundant yellowish ascites'' and ''multiple small whitish nodules'' is correlated with caseous TB appearance in the histological examination in 97% of cases ($P = 0.001$). The results of this statistical analysis are summarized in Fig. 10.

3.5. *The medical treatment*

All the patients with confirmed abdominal TB underwent antimicrobial treatment. The mean duration was 7.2 months (6–10).

4. Discussion

The incidence of the abdominal TB is rising all over the world. Its endemic evolution remains a serious public health problem in many countries.[5] The treatment of this entity should be medical. Many efficient anti-tubercular agents are available. However, anti-TB chemotherapy requires a long follow-up and the rate of side effects is sometimes considerable.[6] Ascites is the most frequent clinical presentation in Tunisia. In that case, the differential diagnosis with peritoneal carcinomatosis is difficult due to the clinical, radiological, and macroscopic similarities.[7] For these reasons, the bacterial confirmation is always needed for the diagnosis. Although the advent of many sophisticated investigations, the abdominal TB remains a diagnostic challenge for all clinicians.

The primary abdominal involvement of TB can be induced by ingestion of the bacteria. However, secondary blood or lymphatic dissemination from lung lesions seems to be more frequent. The bacterial agent is a bovine or human mycobacterium and exceptionally atypical mycobacteria in immune-compromised patients. Abdominal TB remains an affection of young people with no significant predilection for the sex.[8–10]

Gastrointestinal involvement of TB may include tuberculous enteritis, tuberculous peritonitis, and hepatic disease. Rare presentations as pancreatitis or cholecystitis had also been described. Peritoneum and the distal ileum are the most common abdominal locations of TB.[11,12] Our report demonstrated similar epidemiologic findings. The pseudotumoral presentation is frequently reported. This was the case of two patients in our study.[13]

Abdominal pain (66.7%), abdominal distention (60%), and weight loss (52.2%) were the most frequent symptoms in this study. Insidious and non-specific clinical courses are constantly reported. The interval between the onset of symptoms and final diagnosis may exceed 1 year.[14] This induces a clinical polymorphism and increases the doubt. The diagnosis has always to be supported by additional investigations.

Routine laboratory tests have a limited value, especially in this location of TB. The positivity of the tuberculin skin test is not specific for active TB, but simply reflects a prior contact with the TB bacillus. The diagnostic value of this exploration is not considerable according to many authors.[15,16]

Bacterial examination of biological fluids in patients with suspicious abdominal TB is systematic. A high rate of negativity in sputum, urine, and ascites specimens is reported by the majority of series. The probability of a positive acid fast smear may increase with the number of sites sampled.[17]

The radiometric detection method (BACTEC) and nucleic acid amplification techniques as well as adenosine desaminase activity made possible the rapid diagnosis of TB. However, the cost limited the availability of these methods in the routine investigation.[18]

The standard imaging and digestive bariums provided only indirect signs of the disease usually related to effusion or thickening of abdominal organs. However, suggestive signs of pulmonary TB in the chest X-ray are helpful in some difficult case diagnosis.[19]

On a barium follow-through, ileocecal TB can simulate Crohn's disease. Despite being in an endemic area, TB was discussed as a differential diagnosis in some of our patients due to the strong similarity.[20]

The contribution of CT scan is noon debatable. Nodular peritoneum, thickened intestinal wall, and ascites have a high diagnostic value in endemic areas. However, these findings are

never specific. CT remains the gold standard for the diagnosis in the lymph node TB.[21,22]

Except the cases of acute abdomen such as intestinal obstruction or intussusception, which were immediately surgical, all the patients were explored for non-contributive investigations. In our practice, the diagnostic laparoscopy is a gold standard in the investigation of exudative isolated ascites and the positive diagnosis rate of peritoneal TB was up to 98% because of the specificity of laparoscopic findings and the quality of specimens extracted. This method is superior to the CT-guided peritoneal biopsy.[23]

The results of laparoscopic lymph node biopsy are discussible. Laparoscopic management is difficult for some abdominal localization. The high rate of inflammatory lymph node makes multiple biopsies mandatory.

The laparoscopic resection of tubercular intestinal stenosis is feasible. The operative morbidity and recurrence seem to be acceptable in comparison with Crohn's disease.

In the peritoneal and hepatic localizations, laparoscopy allows an exhaustive exploration of the peritoneal cavity and the practice of big biopsies. In these cases, the histopathological examination makes always the right diagnosis. The surgical complications are frequent and reveal the disease in 30% of cases.[24]

In our report, we showed the importance of the surgical exploration in the diagnosis and management of abdominal TB. Anti-tubercular chemotherapy should support the procedures during 6 months.[25]

5. Conclusions

Despite the panoply of available investigations, abdominal TB is the most difficult medical condition diagnosis. The surgery still represents a crucial step in the management. The abdominal laparoscopic biopsy is safe and efficient. In endemic areas, surgical exploration is recommended for each suspicious case.

Conflicts of interest

The authors have none to declare.

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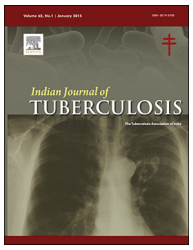

Original Article

Concomitant female genital tuberculosis and endometriosis

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ABSTRACT

Aims: To demonstrate an association between female genital tuberculosis (FGTB) and endometriosis.

Methods: A total of 16 women who underwent laparoscopy (12 cases) or laparotomy (4 cases) and were found to have female genital tuberculosis and endometriosis were enrolled in this retrospective study.

Results: The mean age and parity were 28.2 years and 0.2, respectively. Past history of tuberculosis was present in 75% of the women (pulmonary in 50%). Menstrual dysfunction (especially oligomenorrhoea and dysmenorrhoea), constitutional symptoms, infertility, abdominal pain and lump were the main complaints. Diagnosis of FGTB was made by positive acid-fast bacilli (AFB) on microscopy, culture of endometrial aspirate, positive polymerase chain reaction (PCR), histopathological finding of epitheliod granuloma or findings of TB on laparoscopy or laparotomy. Diagnosis of endometriosis was made by laparoscopy or laparotomy. Pelvic adhesions were seen in all women, whereas frozen pelvis was seen in 7 (43.7%) women. Surgery was performed, which was laparoscopic adhesiolysis in 12 (75%), drainage of endometrioma in 12 (75%), cystectomy in 8 (50%), and total abdominal hysterectomy with bilateral salpingo-oophorectomy in 4 (25%) cases. With more then one type of (surgery in many cases).

Discussion: Female genital tuberculosis and endometriosis may have similar manifestations and can co-exist.

1. Introduction

Tuberculosis remains a major public health problem globally, with 10.4 million new TB cases annually out of which 3.5 are women die.[1] Female genital tuberculosis (FGTB) is common in developing countries causing menstrual dysfunction especially oligomenorrhoea, infertility, chronic pelvic pain, abdominal pain, lump, with or without constitutional symptoms like anorexia, weight loss, fever and night sweats.[2–6] Incidence of

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FGTB was found to be 24.5% in women seeking assisted reproduction in India.[7] FGTB is usually acquired from extra-genital TB, especially pulmonary TB or abdominal TB.[2,3] Fallopian tubes are affected in almost all cases (90–100%) followed by endometrium (50–80%), ovaries (20–30%), cervix (5–15%) and rarely vagina and vulva.[2,3] FGTB is an important cause of infertility in developing countries like India through tubal blockage, peritubal adhesions, abdominal, perihepatic adhesions (Fitz-Hugh–Curtis syndrome), endometrial atrophy and adhesions (Asherman's syndrome).[8–11] FGTB can cause tubo-ovarian masses and masquerade ovarian cancer causing major diagnostic dilemma, as CA-125 may be raised in both conditions.[12] Radiological modalities like CT scan MRI, PET may not be able to differentiate the two conditions.[13,14]

Endometriosis is another common gynecological condition manifesting as chronic pelvic pain, dysmenorrhoea, dyspareunia, infertility and abdominal or pelvic lumps from ovarian endometriomas and adhesions.[15–17] It usually needs laparoscopy for diagnosis and treatment when findings like brown spots, peritoneal defects, endometrioma and adhesions are observed. On laparoscopy in a case of TB, there may be tubercles, localised ascites, caseation nodules, shaggy areas and adhesions.[18] Biopsies can be taken from the representative areas. At laparoscopy, definitive surgery can also be preformed for endometriosis like fulguration of spots, adhesiolysis and drainage of endometrioma.[15–17]

2. Materials and methods

A total of 16 women who underwent diagnostic and operative laparoscopy (12 cases) or laparotomy (4 cases) for infertility, chronic pelvic pain and abdominal masses and were found to have concomitant female genital tuberculosis and endometriosis over the last three years (January 2010–December 2012) in the authors' unit at All India Institute of Medical Sciences, New Delhi were enrolled in this retrospective study. The study was part of an ongoing study on diagnosis and management of female genital tuberculosis, for which ethical clearance of the institute was obtained. Proper informed consent was obtained from all the cases. Detailed history was taken from all women for constitutional symptoms like fever, weight loss, anorexia, night sweats, menstrual dysfunction, infertility, abdominal and pelvis pain and past history of TB. General physical examination for pallor, jaundice, edema, lymphadenopathy, cardiovascular, chest examination, abdominal examination, speculum and bimanual vaginal examination were performed in all women.

All women underwent basic investigations for their infertility and presenting symptoms in the form of complete hemogram, leucocyte count, erythrocyte sedimentation rate (ESR), Mantoux test, urine examination, blood sugar, husband semen analysis, serum Follicle Stimulating Hormone (FSH) and Luteinising hormone (LH). Ultrasound, CT scan or MRI was done in indicated cases. Endometrial sampling was done on menstrual day 21 in all women. One part of the sample was immersed in saline and sent for AFB microscopy, culture and PCR while second part of sample was immersed in formalin and sent for histopathological examination.

All women underwent definitive surgery for diagnosis and therapy depending upon their symptoms, fertility status and findings on laparoscopy and laparotomy. For infertility patients, conservative surgery was performed as far as possible. For endometriosis, drainage of endometrioma followed by removal of cyst wall was done. Adhesiolysis was done to free fallopian tubes and ovaries. Endometriotic lesions were cauterised with bipolar cautery. In few cases, where laparotomy was done for chronic abdominal and pelvic pain and in the absence of infertility, complete pelvic clearance in the form of total abdominal hysterectomy and bilateral adnexectomy was performed as definitive treatment for endometriosis.

All women diagnosed with FGTB were given free antituberculous therapy as per World Health Organisation, Directly Observed Treatment, Shortcourse (DOTS) strategy, under Revised National Tuberculosis Control Programme (RNTCP) of Government of India. It recommends 4 drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) for 2 months followed by 2 drugs (isoniazid and rifampicin) for next 4 months. All women were regularly followed up to ensure compliance. Routine pyridoxine was not given and liver function test were only done if clinically indicated. Women in whom residual lesions of endometriosis were still present after laparoscopic surgery were given GnRH analogues (leupride depot) 3.75 mg sub-cutaneously every 28 days for 3 doses.

The findings on laparoscopy and laparotomy were noted. The data were analysed and appropriate statistical analysis was done using Fisher's exact and chi-square test with p value of <0.05 taken as significant. During the same period, we collected total cases of endometriosis and tuberculosis diagnosed on laparoscopy and laparotomy in the unit to find out the prevalence of co-existent FGTB and endometriosis.

3. Results

The characteristics and presentation of women in the study are shown in the Table 1. The mean age and parity were 28.2 (3.75) years and 0.2, respectively. Past history of TB could be obtained in the 12 (75%) women with pulmonary TB in 8 (50%) women. Menstrual dysfunction was common along with constitutional symptoms. Dysmenorrhoea and dyspareunia were the main complaints seen in 14 (87.5%) women. All women had abdominal pain and mass, whereas 12 (75%) had infertility.

Various methods to diagnose FGTB and endometriosis are shown in Table 2. Positive PCR was present on endometrial sampling in all cases. Findings of FGTB and endometriosis on laparoscopy were seen in all 12 (100%) cases in whom laparoscopy was performed, whereas FGTB and endometriosis on laparotomy were seen in all 4 cases in whom laparotomy was performed. Various surgeries performed are also shown in Table 2; the type of surgery was laparoscopic adhesiolysis in 12 (75%) women, drainage of endometrioma in 12 (75%) women and ovarian cystectomy in 8 (50%) women while laparotomy with total abdominal hysterectomy and bilateral salpingo-oophorectomy was performed in 4 (25%) women.

Table 1 – Characteristics and presentation of women (n = 16).

S. No.	Characteristic	Number	Percentage
1.	Past history of tuberculosis	12	75%
	(i) Pulmonary TB	8	50%
	(ii) Abdominal TB	3	18.7%
	(iii) Bone and joint TB	1	6.2%
2.	Complaints		
	(i) Menstrual problems		
	(a) Menorrhagia	2	12.5%
	(b) Oligomenorrhoea	12	75%
	(c) Amenorrhoea	3	18.7%
	(d) Dysmenorrhoea	14	87.5%
	(ii) Dyspareunia	14	87.5%
	(iii) Fever	16	100%
	(iv) Anorexia	14	87.5%
	(v) Weight loss	14	87.5%
	(vi) Night sweats	14	87.5%
	(vii) Infertility	12	75%
	(viii) Abdominal pain	16	100%
	(ix) Pelvic pain	16	100%
	(x) Abdominal lump	16	100%

Note: Many women had more than one symptom.

Table 2 – Methods used for diagnosis of tuberculosis and endometriosis.

S. No.	Method	Number of cases	Percentage
1.	Endometrial sampling		
	(i) Positive AFB on microscopy	3	18.7
	(ii) Positive AFB culture	4	25
	(iii) Positive PCR	16	100
	(iv) Positive epithelioid granuloma	6	37.5
2.	(i) Positive epithelioid granuloma on peritoneal biopsy	4	25
	(ii) Positive endometriosis on peritoneal biopsy	6	37.5
3.	(i) Laparoscopic finding of genital TB	12	75
	(ii) Laparoscopic finding of endometriosis	12	75
4.	Laparotomy finding of genital TB	4	25
	Laparotomy finding of endometriosis	4	25
5.	Surgery performed		
	(a) Laparoscopy		
	(i) Adhesiolysis	12	75
	(ii) Drainage of endometrioma	12	75
	(iii) Ovarian cystectomy	8	50
	(iv) Cauterisation of lesions	12	75
	(b) Laparotomy		
	(i) Total abdominal hysterectomy with bilateral salpingo-oophorectomy	4	25

Note: Many women had more than one finding.

The various findings of FGTB on laparoscopy and laparotomy are shown in Table 3 and Fig. 1. Apart from tubercles, shaggy areas, beaded tubes, caseous nodules, encysted ascites and tubo-ovarian masses were seen in all 16 (100%) women. Various grades of pelvic adhesions were seen in all women

Table 3 – Laparoscopic and laparotomy findings of genital TB.

S. No.	Findings on surgery	No. of women	Percentage
1.	Beaded tubes	8	50
2.	Tubercles on tubes, ovaries, peritoneum	16	100
3.	Shaggy areas on uterus, tubes and peritoneum	15	93.7
4.	Tubo-ovarian masses		
	(i) Bilateral	6	37.5
	(ii) Right	7	43.7
	(iii) Left	3	18.7
5.	Caseous nodules	6	37.5
6.	Encysted ascites	12	75
7.	Adhesions	16	100
	(i) Pelvic	16	100
	(a) Grade 1	3	18.7
	(b) Grade 2	3	18.7
	(c) Grade 3	3	18.7
	(d) Grade 4	7	43.7
	(ii) Fitz-Hugh–Curtis syndrome	8	50
8.	Frozen pelvis	7	43.7

Note: Many women had more than one finding.

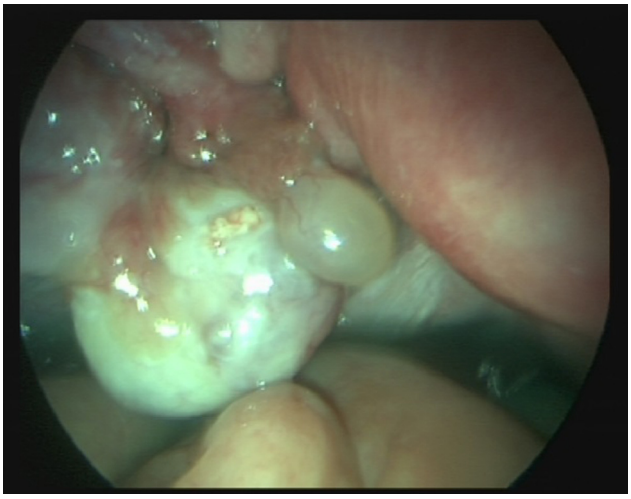

Fig. 1 – Laparoscopic findings of concomitant FGTB and endometriosis showing tubercles, peritubal adhesions and endometriomas.

(16 women, 100%) Fitz-Hugh–Curtis syndrome was seen in 8 women (50%) and frozen pelvis was seen in 7 women (43.7%).

The various laparoscopic and laparotomy findings of endometriosis are shown in Table 4 and Fig. 2. Tubo-ovarian masses with unilateral or bilateral endometrioma were seen in all 16 women. Similarly, pelvic adhesions and frozen pelvis were seen in 16 (100%) and 7 (43.7%) cases, respectively. Other findings included puckered lesion, widow effect and brownish nodules as seen in Table 4. All women were given antituberculous therapy as per Directly Observed Treatment, Short-course (DOTS) strategy, under Revised National Tuberculosis Control Programme (RNTCP) of Government of India and were

Table 4 – Laparoscopy and laparotomy findings of endometriosis.

S. No.	Surgical findings	Number of women	Percentage
1.	Tubo-ovarian mass	16	
	(i) Bilateral	6	37.5
	(ii) Right	7	43.7
	(iii) Left	3	18.7
2.	Puckered lesions	12	75
3.	Window effect	6	37.5
4.	Brownish nodules	16	100
5.	Adhesions	16	100
	(i) Pelvic	16	100
	(a) Grade1	3	18.7
	(b) Grade2	3	18.7
	(c) Grade3	3	18.7
	(d) Grade4	7	43.7
	(ii) Frozen pelvis	7	43.7
	(iii) Fitz-Hugh–Curtis syndrome	8	50

Note: Right fallopian tube was involved more commonly than left tube but not statistically significant (p value 0.8).

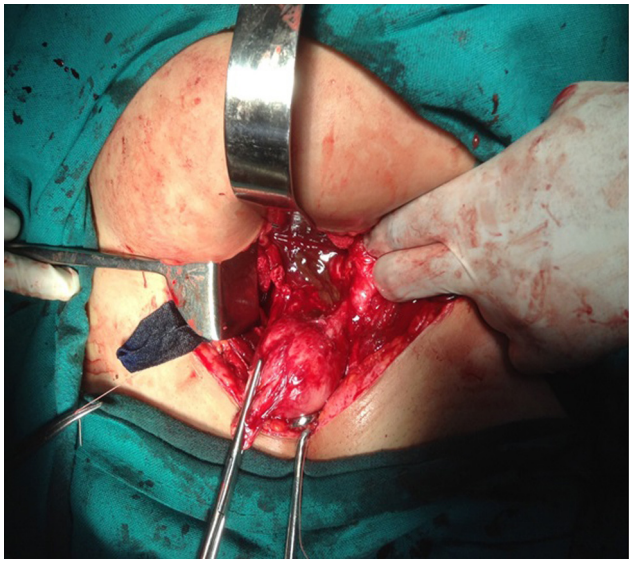

Fig. 2 – Laparotomy findings of concomitant FGTB and endometriosis showing frozen pelvis, chocolate-coloured fluid from endometrioma and dense pelvic adhesions. Total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed.

followed up in the gynecological outpatient department every month. Three women in whom complete surgery for endometriosis could not be performed were given GnRH analogues (leupride depot 3.75 mg sub-cutaneously every 28 days for three injections).

On collection of total cases of FGTB and endometriosis in the unit during the study period, a total of 126 cases of FGTB and 94 cases of endometriosis were diagnosed on laparoscopy and laparotomy. Hence the prevalence of co-existent endometriosis in FGTB was 12.7% (16 out of 126 cases) while prevalence of concomitant FGTB in endometriosis was 17.2% (16 out of 94 cases).

4. Discussion

Female genital tuberculosis is a common disease in developing countries like India and is usually secondary to TB lesion elsewhere in the body spreading through the haematogenous route.[1–4] More liberal immigration and human immunodeficiency virus (HIV) infection have further fuelled the epidemic of TB and emergence of drug-resistant TB.[2] It is an important cause of infertility in India through tubal blockage, peritubal adhesions, endometrial atrophy and adhesions and ovarian involvement.[4–11] It can also present as tubo-ovarian mass and may masquerade ovarian cancer often necessitating laparotomy and frozen section for diagnosis. Positron emission tomography (PET) has been successfully used for diagnosis of tuberculous tubo-ovarian masses and to differentiate them from ovarian cancer.[14] FGTB is a paucibacillary disease and there is difficulty in its diagnosis. Endometrial aspiration or biopsy for AFB on microscopy, culture and histopathological examination for epithelioid granuloma is diagnostic of TB but is present in less number of cases.[2] PCR is a rapid and sensitive method for diagnosis of FGTB, but has high false-positive results and alone cannot be used for diagnosis.[19] Gene Xper has recently been successfully used for diagnosis of FGTB on endometrial sampling with very less false positive rates.[20]

Laparoscopy and hysteroscopy have been used to diagnose FGTB with various findings being tubercles, hydrosalpinx, caseous nodules, adhesions, encysted ascites, white, yellow or opaque plaques over tubes and uterus, tubo-ovarian masses and pelvic and perihepatic adhesions.[18,20,21] Laparotomy is sometimes performed when FGTB cannot be differentiated from ovarian cancer or for pelvic clearance in chronic pelvic pain with mass.[12] However, laparoscopy and laparotomy in FGTB are difficult and are associated with increased complication rate as has been our experience.[22,23] Medical treatment in the form of 6 months of antituberculous therapy with isoniazid, rifampicin, ethambutol and pyrazinamide for 2 months followed by isoniazid and rifampicin for 4 months is needed in all cases of FGTB irrespective of surgery performed.[24] Rarely multidrug resitant FGTB can also be seen necessitating category IV drugs for 2 years.[25]

Endometriosis is another common condition causing infertility, dysmenorrhoea and dyspareunia.[14–16] Definitive diagnosis is made by laparoscopy, which also quantifies the disease through Revised American Fertility Society Classification.[16] Laparoscopy can also be used for management in the same sitting such as adhesiolysis, cauterisation of the spots, cystectomy and laparoscopic uterine nerve ablation (LUNA).[15–17]

Laparotomy and definitive surgery are performed when diagnosis is in doubt or to differentiate it from carcinoma ovary. In case where fertility is not the concern and there is chronic pelvic pain with severe endometriosis, hysterectomy with bilateral adnexectomy can be performed as was done in 4 cases in the present study. We observed co-existent endometriosis with FGTB in 12.7% of the women (16 out of 126 FGTB cases) while concomitant FGTB with endometriosis was observed in 17.2% (16 out of 94 endometriosis cases).

As the prevalence of both FGTB and endometriosis is high, especially in infertility cases, the two conditions can co-exist

without increasing prevalence of each other directly by any definite mechanism explaining the reason of concomitance of the two conditions.

Conflicts of interest

The authors have none to declare.

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Original Article

Biofilm colonization of *Mycobacterium abscessus*: New threat in hospital-acquired surgical site infection

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ABSTRACT

Introduction: Rapidly growing non-tuberculous mycobacteria (NTM) are hazardous cause of post-operative soft tissue infection leading to nosocomial outbreaks following various surgical procedures, especially laparoscopic surgeries using heat sensitive, non-autoclavable surgical instruments.

Methodology: Surgery department of our hospital noticed increase in rate of post-laparoscopic abdominal port site infection (PSI) and informed the Microbiology Department. A prospective investigational study of defined cases with the aim of source tracing and formulation of infection control measures was initiated. Pus or wound scrapings were collected and processed for aerobic, anaerobic bacteria and Mycobacterium, both by staining and culture. Environmental samples were collected from laparoscopic instruments, and different parts of operation theatre (OT). Mycobacterial isolates were speciated by line probe assay. All the cases were treated with clarithromycin and ofloxacin ± amikacin.

Results: Among 15 cases of PSI, 11 patients had undergone laparoscopic cholecystectomy, 3 had laparoscopic mesh hernioplasty and one had laparoscopic orchidopexy. Of the 13 pus/discharge specimens examined, 11 revealed growth of NTM. All the isolates were identified as *Mycobacterium abscessus* by line probe assay. Scraping of biofilm from the disinfectant tray also produced growth of the same organism. Plastic trays used for disinfectants were replaced with metal trays and instructed to do mechanical scrubbing before autoclaving at regular interval. No similar PSI cases were notified after those measures were taken, till date.

Conclusions: This study has shown the need of culture and identification of pathogens causing persistent post surgical wound infections and illuminated importance of rapid source tracing in resource constraint situation which could control outbreak.

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1. Introduction

Non-tuberculous mycobacteria (NTM) are also known as Mycobacteria other than tuberculosis (MOTT) or Atypical mycobacteria due to their opportunistic pathogenesis and saprophytic nature. Previously, they were known for opportunistic infections among immunocompromised patients which do not hold true nowadays. The increase in prevalence of NTM disease has been noted worldwide as well as in India.[1–3] The identification rate of NTM in pulmonary diseases vary from 0.7% to 34%, whereas it is 0.1–27.4% among extra-pulmonary mycobacterial infections in India.[4] NTMs are ubiquitous in environment and are widely distributed in water, soil and animals and they are transmitted by aerosol, soil, dust, water, ingestion or by skin inoculation, whereas human to human transmission is rare.[2] Their ability to produce biofilm helps them to colonize water reservoir, supply line, and drainage water pipe leading to contamination of drinking and hospital water supply and also makes them 1000 times more resistant to disinfectant and antibiotics than free bacteria.[5–8]

Currently, there are more than 125 recognized species of NTM, out of which at least 42 species related with human diseases.[9] Different NTM species especially Runyon group IV, Rapid growing mycobacteria (RGM), are becoming a notorious microbe causing hospital-acquired infections (HAI) in recent times.[1,5,10] Isolated case reports, case series, outbreaks and pseudo-outbreaks of nosocomial RGM infections have come to the press from different parts of India.[11–17] We have investigated the source of laparoscopic port site infections by NTM among cluster of cases and terminated the transmission by appropriate measures. We also have used atomic force microscopy (AFM) as a method to detect biofilms in situ. AFM has an edge over Scanning Electron Microscopy (SEM) for visualizing surfaces of biofilms, as it can act in physiological condition without any pre-treatment of biological sample. Distinctive features of biofilm by different bacteria had been depicted by previous study.[18]

2. Subjects and methods

Department of General surgery of our tertiary care hospital of eastern India, noticed increase in rate of delayed wound healing and persistent discharge from the port site wounds following laparoscopic abdominal surgery and informed the Microbiology Department.

To investigate the cause of port site infection (PSI), **a case definition** was set as (1) the patients who had undergone any abdominal laparoscopic surgery, (2) presenting with delayed wound healing, breakdown of skin after initial healing, discharging sinuses from any of the port site, (3) in spite of one week antibiotic therapy (either amoxycillin-clavulinic acid combination or ofloxacin). All the defined cases were examined for presence of pain, discharge, wound gaping, and fever. Operative history was noted on predesigned proforma. Either pus samples or wound scrapings were collected and processed for aerobic and anaerobic bacteria and Mycobacterium both by staining and culture using blood agar, MacConkeys agar, Robertson's cooked meat broth (RCM), Wilkins Chalgren Anaerobic Agar (HiMedia®, India) supplemented with 5% sheep blood and Lowenstein–Jensen (LJ) medium at 37 °C.

Environmental swab samples were collected from operating table, floor and walls of operation theatre (OT), dressing tray, instrument trolley, sterile gauze, suture material and polypropylene mesh and 2 sets of laparoscopic instruments. We also took 5 mL sample of glutaraldehyde (Cidex) solution in use from the disinfectant tray; 5 mL of tap and sterile water, used for washing hand and rinsing instruments after taking out of sterilant respectively; scraping samples from the glutaraldehyde plastic tray and tap mouth for testing. Swabs, scrapings and centrifuged deposit of liquid samples were examined by Ziehl–Neelsen staining and cultured on LJ media.

Aerobic bacterial isolates were identified by routine laboratory methods and Mycobacterial isolates were tested with TB Ag MPT64 Rapid test (SD Bioline, Seoul, South Korea) and speciated by line probe assay using GenoType Mycobacterium CM/AS (Hain Lifescience GmbH, Nehren, Germany).

All the NTM positive cases were treated with clarithromycin and ofloxacin ± amikacin for three months and one persistent case needed surgical debridement along with six months of antibiotic therapy.

The procedure followed for sterilization in OT was supervised by monitoring packaging, transport of OT dresses to central sterilization, autoclave sterility cheque and concentration, pH, frequency of change of disinfectant, contact time with instruments and also scrubbing of surgeons.

Plastic trays used for disinfectant were collected and examined by AFM in the tapping mode to detect the laser scanned surface topography of biofilm nature.[18] The height images were analyzed using the WSxM software.

3. Results

Among 15 cases of PSI presented in the OPD during month of February, 2015, 11 patients had undergone laparoscopic cholecystectomy, 3 had laparoscopic mesh hernioplasty and one had laparoscopic orchidopexy during previous six months (August, 2014–January, 2015). A total of 31 port sites of 15 cases were recorded to have painless wound dehiscence with chronic sero-sanguinous discharge (Fig. 1). The wounds were healed initially after surgery over 1–2 weeks. Then indurations appeared at port sites followed by swelling which subsequently ruptured to form sinus. The average interval between surgery and discharge from port site was 28–35 days and epigastric and umbilical ports were commonly affected. No patient was reported to have any fever or systemic complications. Of the 15 cases, pus/discharge specimens could be collected from 14 patients, among which 10 were positive for acid-fast bacilli (AFB) by Ziehl–Neelsen staining and 11 revealed growth of NTM. All the NTM isolates were identified as *M. abscessus* by line probe assay. Mixed bacterial infection was found in 3 cases (*M. abscessus* with *Staphylococcus aureus*/*Pseudomonas* spp.). Scraping from the disinfectant tray also produced growth of the same organism.

After every surgery, laparoscopic instruments were used to wash with tap water, air-dried and dipped in glutaraldehyde (2% solution) for 30 min followed by washing with sterile water and drying. Three plastic trays were placed for disinfectant

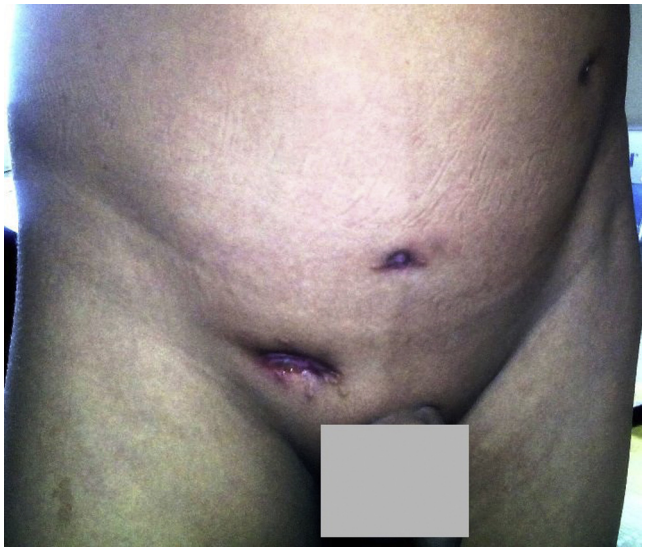

Fig. 1 – A case of port site infection following laparoscopic orchidopexy with sero-sanguinous discharge.

which was replaced with freshly prepared glutaraldehyde solution after every 10th day as per manufacturer's manual. Disinfectant trays were in use for 2 years and visible biofilm present on inner surface. Tray surface was positive for 3-dimensional height images of biofilm by AFM study (Fig. 2).

4. Measures taken following study

Plastic trays used for disinfectants had been replaced with metal trays and instructed to do mechanical scrubbing before autoclaving at regular interval (Fig. 3). Practice of using tap water for rinsing of soiled instruments changed to sterile water. New disinfectant 0.55% orthophthaldehyde (OPA) instead of 2% glutaraldehyde had been introduced.

No similar PSI cases were notified after the measures taken during six months of follow-up period.

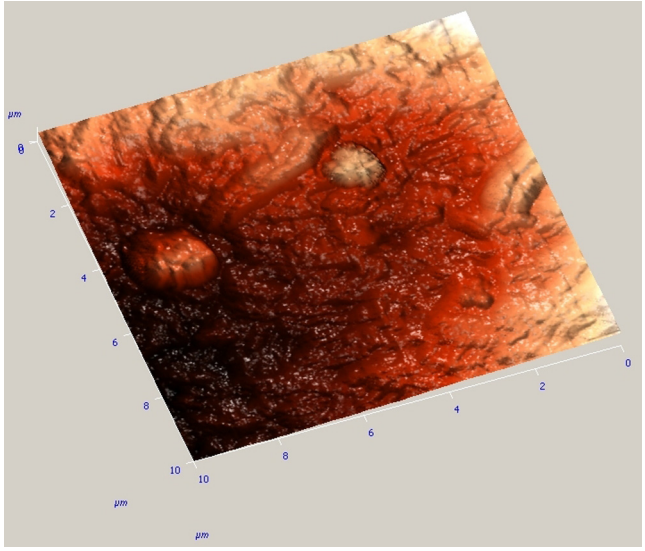

Fig. 2 – AFM image of biofilm on the inner surface of disinfectant tray in use taken at 10 µm × 10 µm scan in the tapping mode.

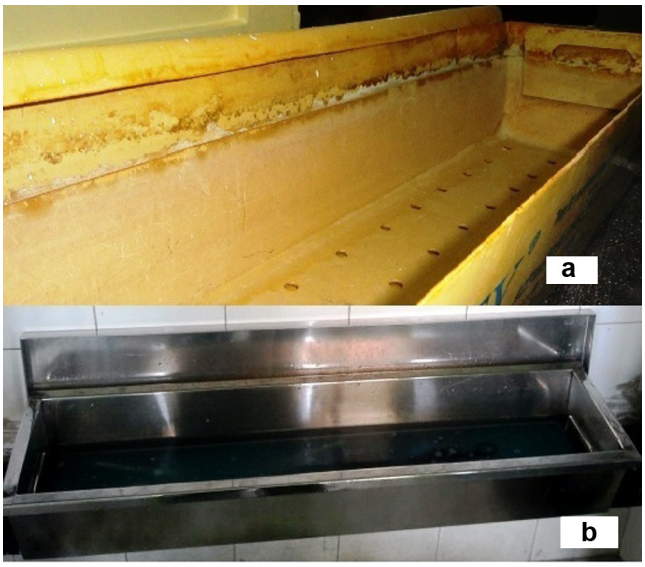

Fig. 3 – Old plastic trays (a) used for disinfectants have been replaced with metal trays (b).

5. Limitations of the study

Epidemiological typing and antibiogram of the *M. abscessus* isolates could not be done due to limited resources.

6. Discussion

A wide spectrum of post-surgical infections by RGM have been identified from endocarditis, endophthalmitis, cellulitis to delayed wound healing following varieties of surgical procedures like cardiac valve replacement, percutaneous balloon angioplasty, sternotomy, rhinoplasty, liposuction, mamoplasty, ophthalmic operations, epidural injection and even minor punch biopsy. Use of contaminated equipments, syringes, prosthetic devices, vaccine, drugs, reuse of single-use devices and contaminated water source resulted in NTM outbreaks in hospitals.[5,10,19–21] Laparoscopic surgery is more vulnerable than open surgery for this infection because the critical instruments used are heat-sensitive, non-autoclavable and sterilized commonly by chemical disinfectants. Organic matter and water carried by the instruments alter the strength of disinfectant which could not attain even high-level disinfection. Therefore, instruments carry microbes and implant them inside soft tissue of abdomen they come in contact with.

Though *M. fortuitum* is the most common RGM reported causing nosocomial postsurgical infections in India,[12–14,17,19] few outbreaks by *M. abscessus* also came into light.[15,22] Both of these species are extremely tough and highly resistant to various disinfectants, not surprisingly making them the most frequent cause of HAIs. Now, *M. abscessus* pose a real threat

due to high-level resistance to available antibiotics and require long-term combination therapy.[23] In our study, one case, non-responsive to combination therapy (clarithromycin and ofloxacin ± amikacin) for three months, needed surgical debridement though we have not tried intra-lesional amikacin injection suggested by a previous study.[24]

Contamination of hospital water supply with RGM has been designated as source of outbreak by previous studies; thus, use of sterile water in spite of tap water for washing of invasive instruments has been suggested by IDSA guideline.[5,6,25] Though we could not isolate the pathogen from hospital water source, might be due to lack of liquid culture system, but presence of biofilm documented by AFM surface topographic study and isolation of the same species from scraping of disinfectant tray delineate that biofilm must be potential constant source of planktonic bacteria which contaminate instruments. When sampling a potential source for NTM colonization, biofilm should also be included in the samples as NTM is good biofilm producer. Biofilm also renders them protection from killing by disinfectant, thus only maintaining proper concentration, pH and adequate contact time with high potency disinfectant might not be sufficient to kill them.[26] Using metal surface which has more anti-biofilm property than plastic and mechanical removal of biofilm regularly before heat sterilization could be a weapon in resource-limited setting. Use of OPA, with wider active pH range, more mycobactericidal property and lesser contact time requirement rather than glutaraldehyde was also an effective measure when ethylene oxide sterilization is not available.

Future studies are needed to show the bio-filming capability of these NTM isolates by in vitro study and effect of different disinfectants on the biofilm which was beyond scope of present study, as we focused mainly on prompt infection control measures to stop transmission.

Our thorough investigation and timely interventions not only helped to formulate proper treatment regimen, but also identified the source and illuminated importance of customized infection control policies to check transmission and HAI outbreak situation.

Conflicts of interest

The authors have none to declare.

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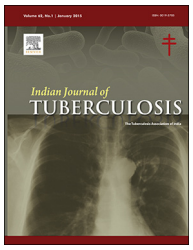

Original Article

Diagnosis of tuberculous meningitis: Current scenario from a Tertiary Neurocare Centre in India

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ABSTRACT

Background: Tuberculous meningitis (TBM) is a condition that is caused by *Mycobacterium tuberculosis* complex and is difficult to diagnose due to the nonspecificity of the presentations. The study analyzed the different modes of diagnosis available in a developing country set up over a period of five years to understand the diagnostic values of the current conventional and automated methods of diagnosis of TBM among the patients suspected with chronic meningitis.

Methods: A total of 10,281 cerebrospinal fluid samples (CSF) were collected from suspected chronic meningitis patients, of which 790 samples were from individuals who had clinically suspected TBM. The samples were subjected to CSF cytology and staining, culturing, immunological tests, molecular techniques, and methods for detection of drug resistance.

Results: The TBM patients were predominantly male, with a mean age of 21–40 years. CSF pleocytosis and lymphocytic predominance were noted. Culture had 40.13% positivity among clinically suspected TBM patients. The multidrug-resistant *M. tuberculosis* (MDR-TB) constituted 3.14% of the total clinical isolates. With IS6110 PCR, a specificity of 92.86% and sensitivity of 100% are seen with an assay threshold of 30 pg/ml. Line probe assay (LPA) using culture isolates had a sensitivity of 97.67% and a specificity of 100%. Direct CSF LPA showed a sensitivity of 96.15% and a specificity of 100%.

Conclusions: A combination of techniques that involved culture, cytology, and DNA amplification methods was found to be promising in specific, accurate, and rapid detection of *M. tuberculosis* in the CSF samples from patients.

1. Introduction

Tuberculous meningitis (TBM) is a type of chronic meningitis, which involves the inflammation of meninges and the subarachnoid space that lasts for 4 weeks or longer.[1] It usually has subacute onset and persisting cerebrospinal fluid (CSF) abnormalities lasting for at least one month. Specific diagnosis of chronic meningitis is difficult just by clinical evaluation due to the diversity of the possible cause and the nonspecificity of the presentations. TBM, caused by *M. tuberculosis* complex, is one of the leading causes of chronic meningitis in the Indian

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0019-5707/

population. India is the highest TB burden country in the world, accounting for one-fifth of the global incidence. In 2015, 10.4 million people fell ill with TB and 1.8 million died from the disease (including 0.4 million among people with HIV). Over 95% of TB deaths occur in low- and middle-income countries.[2]

The CNS disease is an uncommon yet highly devastating manifestation of tuberculosis and carries a high mortality and a distressing level of neurological morbidity, and disproportionately afflicts children and HIV-infected individuals. A 2013 study observed that CNS TB ranged from 0.5 to 2% in all TB patients from different demographic groups, and it accounts for 5–10% of extrapulmonary TB.[3] The CNS tuberculosis exhibits a protean nature, is relatively rare, and its burden lies largely in the resource-starved third-world countries of the world.

This 5-year study attempts to evaluate the efficacy of the different diagnostic approaches for rapid detection of TBM. Our hospital is one of the largest neuroscience centers in the region that caters to central nervous system (CNS) diseases of the entire population of the country. Large numbers of CSF samples are investigated in the center for different conditions. Therefore, this center offers an excellent premise for this study. CSF analysis is the most important parameter for the diagnosis of infectious meningitis and acts as an excellent sample for the diagnosis and study of CNS infections and neurological disorders.

2. Methods

The study was carried out during the period from November 2009 to 2014 using the CSF samples from patients clinically suspected to have TBM/chronic meningitis. The study was approved by the Institute Ethics Committee of the study center and the following criteria were followed for inclusion of the samples in the study:

Inclusion criteria for CSF:

- Fever, headache, and/or meningeal signs lasting for more than 14 days.
- Vomiting, alteration of sensorium, or focal deficit.

Exclusion criteria for CSF:

- The samples from patients showing clinical signs of acute meningitis/immediate onset.
- Known patients of carcinomatous meningitis and degenerative neurological disorders.

The different tests performed to arrive at a tubercular etiology were the following: Mycobacterial culture by conventional and automated method, CSF cytology and staining, IS6110 polymerase chain reaction (PCR), and line probe assay (LPA). The drug susceptibility testing (DST) was carried out using the Mycobacterium growth indicator tube (MGIT) automated system (BACTEC MGIT 320 from BD, India).

Lowenstein-Jensen's medium (LJ) was used for the detection of *Mycobacterium tuberculosis* complex by conventional culture with weekly inspection. MGIT automated system was also used to culture the mycobacteria from CSF samples. Once the growth appeared, a Ziehl-Neelsen (ZN) stained smear was examined to confirm the presence of the bacilli. The differentiation between tuberculous and non-tuberculous mycobacteria was done using a rapid immunochromatographic identification test that uses mouse monoclonal anti-MPT64.

The CSF cytology involved the cytospin smear preparation and staining by Leishman's stain. The presence of floating granuloma was considered to be a suggestive observation.

Uniplex PCR targeting the IS6110 region of the mycobacterial DNA was standardized for determining the presence of *M. tuberculosis* in the patient's CSFs and in the corresponding liquid cultures. Isolation of Genomic DNA was carried out with the method described by Deshpande et al.[4] that uses the cetyl trimethyl ammonium bromide (CTAB) – NaCl and chloroform: isoamyl alcohol extraction technique.

The primers used were of the following sequences[5]:

Forward: 5′ CGT GAG GGC ATC GAG GTG GC 3′

Reverse: 5′ GCG TAG GCG TCG GTG ACA AA 3′

A 50 μl reaction mixture contained a final concentration of 1× assay buffer, 200 μM each of dNTP's, 0.25 μM of each primer, 2.5 U/μl of Taq DNA Polymerase, and 5 μl of extracted DNA. The reaction was amplified for 35 cycles, and the cycling parameters for the reaction were as follows: 94 °C for 30 s for denaturation, 55 °C for 30 s for annealing, and 72 °C for 45 s for extension.

The antimycobacterial drug susceptibility testing was also carried out using the BACTEC MGIT 320 system to the four front-line drugs (SIRE) – Streptomycin, Isoniazid, Rifampin, and Ethambutol according to the manufacturer's guidelines.

The LPA technique involved genotyping of *M. tuberculosis* using nitrocellulose strips containing probes for the genes that code for the rifampicin and isoniazid resistance in *M. tuberculosis* complex. The test was carried out using the GenoType MTBDRplus version 2 (Hain Lifesciences, Germany), which allows detecting the presence of *M. tuberculosis* complex from smear negative pulmonary samples and cultures from pulmonary and extrapulmonary samples. The test has not been validated for detection from direct CSF samples.

The study attempted to standardize the LPA for getting results from direct CSF samples. For this purpose, culture-positive CSF samples were tested along with the corresponding liquid culture. $H37R_V$ was used as the positive control, while distilled water was used as negative control.

3. Results

A total of 10,281 CSF samples were received in the laboratory for chronic meningitis work-up. Of these, 790 (7.68%) CSF samples were from individuals who had clinically suspected TBM based on the clinical findings. These cases, therefore, were taken up for further evaluation.

3.1. *Demographic findings*

Among the 790 patients, there were 520 males (65.82%) and 270 females (34.18%). The patients were in the age group ranging from 2 to 86 years with a mean age of 31.98 years (SD ± 13.81). Majority of patients of this population (30%) belonged to the

age group of 31–40 years. There were 49 (6.2%) pediatric patients with ages ranging from 2 to 10 years. Among this group, there were 27 (51.10%) males and 22 females (48.90%).

3.2. *Laboratory findings*

CSF pleocytosis and lymphocytic predominance were the predominant features observed in patients of TBM. In the case of TBM, the mean cell count was 161.5 cells/cumm. CSF cytospin and staining could suggest a diagnosis of TBM in 20.40% of the patients of chronic meningitis. Floating granuloma (tubercles) was one of the characteristic observation in TBM. Using the cytospin technique to improve the demonstration of the inflammatory cells in order to obtain accessory evidence added to the diagnosis. The characteristic feature often noted in the case of TBM is the 'floating tubercles' along with reactive monocytes. These floating tubercles are clumps of reactive monocytes with mononuclear cells such as small lymphocytes and polymorphonuclear cells. Sometimes, giant cells that are multinucleated inflammatory cells were also seen.

Culture is the gold standard for TBM diagnosis, and a total of 317 culture-positive cases (40.13% with MGIT and/or LJ culture) were observed among clinically suspected TBM patients. On comparison – data was available for 231 samples for which both the cultures were put up simultaneously (Table 1) – MGIT had a higher recovery rate (90.48% – $n = 209$) than LJ media (35.93% $n = 83$). However, the conventional system could exclusively recover the bacilli from 9.52% samples. Among the samples that were cultured on both MGIT liquid system and LJ solid media, the liquid culture had more than twice the recovery rate than solid culture.

In this study, antimycobacterial drug susceptibility testing (DST) was carried out for 191 isolates, majority of which (76.96%) turned out to be pan-sensitive to the first-line drugs (SIRE); 7.85% and 5.24% showed monoresistance to isoniazid and streptomycin, respectively. Multidrug-resistant (MDR-TB) isolates constituted only 3.14% of the total clinical isolates (Table 2). No monoresistance to rifampicin was observed.

PCR was carried out with all 790 CSF samples (Table 3). Among the culture-confirmed patients, all the CSF samples (100%) gave a positive IS6110 PCR result, thus confirming the presence of mycobacterial DNA in the samples (Fig. 1). Additionally, IS6110 PCR detected the presence of mycobacterial DNA in 68.92% ($n = 326$) of the CSF samples belonging to the culture-negative group ($n = 473$). A total of 81.39% IS6110 PCR positivity ($n = 643$) was observed in the overall clinically suspected TBM group. The IS6110 PCR was done for a group of controls ($n = 105$) to assess the sensitivity and specificity of the test (Table 4). The group was classified as non-TB infections, non-infectious conditions, and TB culture-confirmed cases. The non-TB infection controls included culture-positive CSFs from cryptococcal meningitis ($n = 20$) patients and Gram-negative bacterial meningitis ($n = 15$) patients. The non-infectious controls included CSF from patients with neurodegenerative disorders ($n = 20$) and malignancy ($n = 15$). Of these, 4 samples from non-TB infection controls and 1 from the non-infectious controls gave a positive IS6110 PCR. A specificity of 92.86% and sensitivity of 100% were seen with a diagnostic accuracy of 95.24%. The results were found to be statistically very significant ($P < 0.001$). Assay had a threshold of 30 pg of *M. tuberculosis* complex DNA per milliliter.

A total of 92 tests were carried out using the LPA kit GenoType MTBDR*plus*, which included 90 test samples and

Table 1 – Comparison of conventional and automated methods among TB culture positives ($n = 231$).

TB culture result	No of samples
LJ positive; MGIT negative	22 (9.52%)
LJ negative; MGIT positive	145 (62.77)
LJ and MGIT positive	61 (26.41%)
LJ contaminated with MGIT positivity	3 (1.30%)
Total	231

Table 2 – The Streptomycin, Isoniazid, Rifampicin, and Ethambutol (SIRE) drugs susceptibility pattern observed in the study.

Serial No.	SIRE susceptibility pattern	No. of samples
1	SSSS	147
2	SRSS	15
3	RSSS	10
4	SRSR	7
5	SRRS	4
6	RSSR	2
7	SSSR	2
8	RRSS	2
9	SRRR	1
10	RRRS	1
Monoresistance distribution		
1	Streptomycin	5.24%
2	Isoniazid	7.85%
3	Rifampicin	0%
4	Ethambutol	1.04%

Table 3 – Performance of IS6110 PCR in comparison to TB culture.

	Total TB study population		
Culture result	PCR positive	PCR negative	Total
Positive	317 (100%)	Nil (0%)	317
Negative	326 (68.9%)	147 (31.08%)	473
Total	643 (81.39%)	147 (18.61%)	790

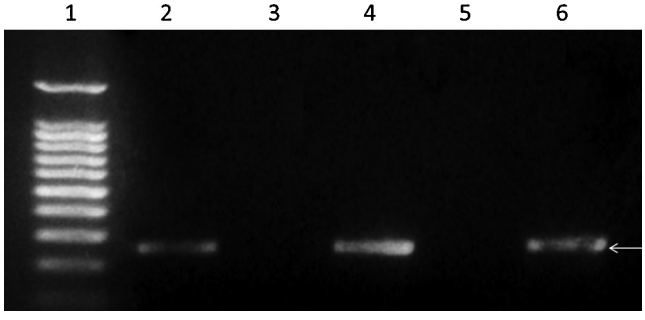

Fig. 1 – The agarose gel image representing the IS6110 PCR positive samples (Lanes 2 and 4). Lane 1 shows 100 bp DNA ladder, Lanes 3 and 5 had the negative controls, while Lane 6 had the positive control ($H_{37}R_v$).

Table 4 – Performance of IS6110 PCR in the control population.

Positive controls: TBM cases			
Control cases	PCR positive	PCR negative	Total
TB culture confirmed	35 (100%)	Nil	35
Negative controls: non-TB infection cases			
Control cases	PCR positive	PCR negative	Total
Cryptococcal meningitis	3 (15%)	17 (85%)	20
Gram-negative bacterial meningitis	1 (6.67%)	14 (93.33%)	15
Negative controls: non-infectious cases			
Control cases	PCR positive	PCR negative	Total
Guillain–Barré Syndrome	1 (5%)	19 (95%)	20
Malignancy-associated meningitis	Nil	15 (100%)	15
Total	6	65	105

2 negative controls. The test samples included 45 CSF samples, which had earlier given a positive culture isolation of *M. tuberculosis* and 45 of the corresponding culture isolates. The negative controls were an *E. coli* clinical isolate and an MGIT culture-negative CSF sample. Drug susceptibility testing was carried out for two of the first-line anti-tubercular drugs, namely isoniazid and rifampicin (Fig. 2, Table 5). After the performance of the test, it was noted that one or a combination of the 3 control bands were missing in the case of 21 test strips, and these were considered as invalid results, thereby giving a total of 71 valid results. A consistency in result was noted between the direct CSF samples and their corresponding cultures, showing sensitivity toward both isoniazid and rifampicin. Additionally, 14 of the isolates from CSF cultures (hereafter mentioned as culture samples) also showed sensitivity toward both the drugs. Among the 21 invalid tests, 19 were of direct CSF samples and the rest were of culture. Among the cases that were shown to have isoniazid and rifampicin resistance, one sample and its corresponding culture and 2 other culture samples were included. Of the 2 corresponding CSF samples of the latter, one showed inconsistency in result and the other showed an invalid result. Isoniazid resistance with rifampicin sensitivity was observed in 4 of the CSFs samples and their corresponding cultures. Also, 2 other culture samples exhibited this pattern of susceptibility while no valid test result was observed in the case of their corresponding CSF samples. The test using culture samples had a sensitivity of 97.67% and a specificity of 100%. When the data from the direct CSF LPA was analyzed, a sensitivity of 96.15% and a specificity of 100% were observed.

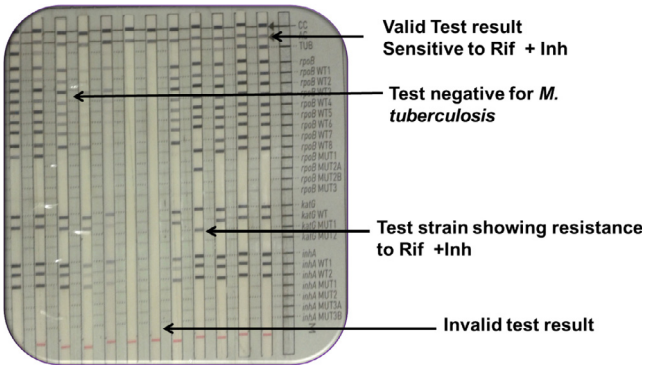

Fig. 2 – LPA strips showing the different test results observed during the study. The different susceptibilities toward rifampicin (Rif) and isoniazid (Inh) drugs are shown.

4. Discussion

Chronic meningitis affects about 10% of the patients diagnosed with meningitis,[6] and in a resource poor setting like that of India, it is highly necessary to have a working knowledge about the prevalence and other epidemiological aspects of the disease as well as to develop diagnostic and treatment strategies that cause the minimum possible burden on the patient and the country.

Table 5 – Results of LPA test performed on direct CSF samples and their corresponding culture isolates.

Susceptibility pattern	From culture	From direct CSF sample	Inconsistency b/w culture and CSF	CSF-test invalid	Culture-test invalid
Isoniazid+ Rifampicin: Sensitive	34 (79.07%)	20 (80%)	Nil	16	2
Isoniazid+ Rifampicin: Resistant	3 (6.98%)	1 (4%)	1	1	Nil
Isoniazid: Resistant; Rifampicin: Sensitive	6 (13.95%)	4 (16%)	Nil	2	Nil
Total	43	25	1	19	2

Only 5–10% of the individuals exposed to *M. tuberculosis* develop TB, and up to 70% of those who do develop the disease are male.[7] It was found that a majority of the patients in this study were male, which was the case with many studies throughout the globe, including India. WHO's report on 'Gender & Tuberculosis' states that incidence rates are higher for males at all ages except in childhood where rates are higher in females.[8] This gender bias is poorly understood, and the under-reporting of infections among women, especially in the developing countries owing to the social set-up, could be one reason why the percentage incidence in women is as low as 30%. Also, men are less likely than women to complete the TB treatment, although they have better access to treatment than their female counterparts, mostly due to the pressure of earning a livelihood. This often leads to disseminated TB infections including TBM.[8] An earlier report that studied the host immunity regulation by the host's gonadal steroids can be extrapolated to state that the endocrine system has a definite role to play in the pathogenicity of TB and could be one of the contributors to this gender disparity among TB patients.[9] However, much more research is required in this regard to explain this phenomenon in *M. tuberculosis* infections. There have been several studies that have attempted to check the age correlation between TB and age. When compared to the western world where mostly the elderly are affected, the Indian scenario is shifted toward a younger, more active population within the range of 25–50 years,[10] as seen in this study.

Usually in TBM, the CSF cell count is seldom above 1000 cells/mm^3. However, in our study, we found 2.66% of such cases in the total study population with suspected TBM. Although there is a general consensus on the finding of a pleocytic lymphopredominant CSF, the reports vary in the case of the number and range of cells.

A report published in 2007 from this study center mentioned that the conventional culture using LJ medium has a recovery rate that varies from 42 to 77%. The same study, based on their experimental results, reported a 39% recovery rate from CSF samples.[11] Recovery rate using cultures can be increased by processing multiple specimens from each patient or by increasing the volume of the CSF sample, although published studies mention that around 20% of TBM cases still would not give a positive culture result.[12] In this study, it was noted that a single culturing technique alone could not detect all the positives in the population. According to Somoskovi et al., the non-recovery of the bacilli from all or some of these samples by the liquid culture could be due to isolates' inability to metabolize the palmitic acid in the modified Middlebrook 7H9 broth medium.[13] WHO also has mandated that all specimens cultured on liquid media also should be inoculated on solid media.[14] Demonstration projects conducted by the Foundation for Innovative New Diagnostics (FIND), in association with WHO, have shown that liquid culture and drug susceptibility systems are feasible for implementation in lower income settings to improve diagnosis of smear-negative cases especially in case of suspected central nervous system tuberculosis.[14]

The main hurdle in rapid demonstration of the tubercle bacilli is its slow growth rate. The molecular diagnostic tests are considered to be an answer to this problem and many diagnostic tests have been proposed that involved the amplification of specific gene targets in the *M. tuberculosis* DNA as reviewed by Takahashi et al.[15] For this study, we targeted the IS6110 gene sequence, which is an insertion element that is found exclusively within the *M. tuberculosis* complex, and therefore an important diagnostic marker in differentiation of *M. tuberculosis* complex from the other species of mycobacteria.[16] The high sensitivity of the PCR assay makes it an ideal diagnostic tool for the rapid detection of *M. tuberculosis* complex in CSF of the TBM-suspected patients. Some of the earlier studies on this assay were carried out using a smaller number of CSF samples[17,18] or on multiple clinical samples.[5] However, this study was carried out on a large CSF sample size, which makes the study more valid and be a reflection of the actual incidence. One statistical challenge in assessing these tests is the low acknowledged sensitivity of the gold standard. As a result, the specificity calculation may not reflect the actual diagnostic value of the test in question.

When the performance of the PCR test was evaluated in the control population, the non-infectious controls showed a higher degree of negativity for IS6110 target than when compared to non-TB infectious samples. The *M. tuberculosis* strains typically contain multiple copies of IS6110 (up to 25 per genome), but those with only a single copy or no copies have also been identified.[19] The high variability in copy number and location of IS6110, as well as its stability over time, renders IS6110 a useful diagnostic and epidemiological tool. Additionally, in certain strains, the location of a copy can be specific, thereby allowing its usage for the rapid identification and differentiation of particular strains from other isolates.[20]

The GenoType MTBDR*plus* assay is designed to simultaneously detect the most important gene mutations conferring rifampicin (*rpoB* genes) and isoniazid (*inhA*, *katG*) resistance in *M. tuberculosis* isolates within 8 h[21] and is also recommended by RNTCP (Govt. of India).[22] However, the test has not been standardized for the extrapulmonary samples to the best of our knowledge. This study made an attempt to standardize the protocol for use with direct CSF samples in order to rapidly diagnose drug resistance among TBM causing strains.

Although the sensitivity and specificity of the test were statistically found to be high, the number of test runs was low, and therefore, more number of samples needs to be studied to make a reliable conclusion in this regard. Also, the outcome of the study is dependent on the bacilli load in the CSF, which is generally paucibacillary. In this study, as the standardization required the prior knowledge of presence of bacilli in the samples, we tested samples that gave a culture confirmation. All of these samples were also IS6110 PCR positive. Presently, performance of the test with culture-negative CSF samples remains unanswered. However, when clubbed with the PCR study, it can be assumed to be a good predictor of the drug resistance pattern exhibited by the isolate. Further study and standardization in this direction can help minimize the time required for diagnosis by weeks. Additionally, the cost for first isolation and then the DST can be reduced by this one-step processing, which gives combined data on presence of the *M. tuberculosis* complex and its drug resistance pattern. The DST on the other first- and second-line drugs would be undertaken in the next phase of the study.

5. Conclusion

The study analyzed a large number of CSF samples that can be considered a unique feature when attributed to a single study center in the country. It shows that no single test is enough for the diagnosis of TBM. Molecular methods like IS6110 PCR and Line probe assays can be standardized to achieve rapid diagnosis of TBM. The LPA tests also help in rapid detection of drug resistance among the TB bacilli, and also in the long run helps in reduction of total diagnostic costs for the patient. Future studies can be directed with a larger study population. Also, in order to better understand the demographic and other epidemiological factors involved in the progress and prevalence of infection, study can be carried out in various populations and correlated with clinical observations.

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Authors' contribution

JEK was involved in preparing the study protocol, sample collection, laboratory analysis, and calculation of results and interpretation of results. RR was involved in conceiving and designing the study. He was involved in supervision of the study and analysis and interpretation of results. Both the authors contributed to the preparation of manuscript and the final manuscript was approved by both authors.

Conflicts of interest

The authors have none to declare.

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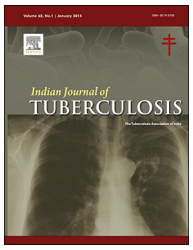

Original Article

Factors affecting applicability of "*home-based interventional model*" for active case finding among household contacts of index cases of pulmonary tuberculosis in Kashmir

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ABSTRACT

Background: There are various biological, behavioural and social determinants associated with the epidemiology of pulmonary tuberculosis (P-TB) which have been extensively described in literature. Present study was conducted to know the influence of such factors on the "*home-based interventional model*" for active case finding among household contacts of index P-TB cases.

Methods: In a quasi-randomized controlled interventional study, the "*home-based interventional model*" for active case finding was applied to index P-TB cases and their household contacts of *interventional group* of study subjects. Study was conducted from January 2014 to June 2015 for evaluation of the model in comparison to the existing usual practice of contact tracing. Baseline data regarding factors associated with epidemiology of P-TB likely to influence effectiveness of intervention were collected, collated and analysed.

Results: The overall acceptance of intervention was 70.74% which was statically significantly influenced by gender ($p < 0.0001$), age group ($p = 0.0006$), education ($p < 0.0001$), occupation ($p < 0.0001$) and relationship of household contacts with index case ($p < 0.0001$). Total 27 (4.51%) cases were detected among contacts. Out of personal and family level characteristics "relationship with index case" ($p = 0.0418$) and "overcrowding in house" ($p = 0.0017$) were found to statistically significantly influencing the effectiveness of interventional model. The prevalent risk factors for TB among contacts, level of exposer of household contacts to index cases, grade sputum positivity and type of index case were not found statistically significantly influencing the yield of *interventional model*.

Conclusion: Factors/determinants influencing the applicability of interventional model for active case finding among household contacts were identified and their impact on acceptance and yield of intervention was assessed.

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1. Introduction

Globally, tuberculosis (TB) is a leading cause of death.[1] It is a major public health problem worldwide, especially in developing countries including India.[2] Tuberculosis has severely affected communities and nations since times immemorial. The disease has brought untold miseries to generations and even today, when newer modalities for diagnosis and treatment of TB have made the disease curable, people are suffering and dying from the disease.[3] Every person in the community is at risk of TB infection because it is an airborne infection from sputum smear positive TB patients when they speak, sneeze and cough. The risk of infection depends on the concentration of the expelled bacilli from the patient, the level of ventilation in households and the duration of exposure of the uninfected individual to the patient.[4] Despite dramatic improvements made since the 1990s in providing access to high-quality TB services, many people with TB remain undiagnosed or are diagnosed only after long delays.[5]

During the past few years, there has been an intensified discussion about using active case-finding as a possible complement to the predominant approach of ''passive case-finding''. The primary objective of such active case finding or screening is to ensure that active TB is detected early to reduce the risk of poor disease outcomes and the adverse social and economic consequences of the disease, as well as help reduce TB transmission.[6] Active case-finding involves actively locating, diagnosing, treating and following up with TB patients in communities. This method of identifying TB patients enables household members to be placed on TB treatment if they are found to have the disease. Household contacts are defined as those who share a bedroom, kitchen, bathroom or sitting room with the index case.[7]

In India, RNTCP Operational Research agenda of 2013 included a research topic titled ''*To Design & Evaluate Interventions for Active Case Finding in High Risk Groups (Clinically and socially vulnerable population)*''.[8] In this context we have designed following ''*home-based interventional model*'':

> ''Active case finding among household contacts (≥10 years of age) at home of patient with pulmonary-tuberculosis (P-TB), by medical officer, on pre-fixed & pre-informed *weekly contact evaluation day*, by using sputum microscopy.''

The present article is about the study of factors affecting applicability of this *model* for active case finding among household contacts of P-TB index cases in Kashmir.

The factors studied in present study can be broadly classified as (a) biological determinants: sex, age and immune status (b) behavioural determinants: relationship between household contacts and index case; occupation; smoking, alcoholism and drug addiction; (c) social determinants: socioeconomic class, social category and housing conditions (d) characteristics of index case in relation to power of spreading infection and (e) level of exposer of household contacts to the index case.

2. Material and methods

After designing the ''*home-based interventional model*'' for active case finding (as described above), two TB Units – Uri from northern and Shopian from southern Kashmir, were randomly selected using computer software. The basic study design was *quasi-randomized controlled interventional study* in which the index P-TB cases in each selected TB Units were allocated to *test* (Interventional) and *control* groups by ''alternate assignment'' method. Study was conducted to evaluate the home-based interventional model in comparison to usual RNTCP practice for contact tracing. *Present article is limited to the secondary objective of study about the factors influencing the acceptance and yield of the model.*

The proposal for study was approved by the *Ethical Review Committee* of GMC Srinagar and *State RNTCP Operational Research Committee*. The permission for the study was obtained from *State Tuberculosis Officer Kashmir*, who also directed DTOs and MOTCs of concerned TB Units to provide logistics and technical support for sputum microscopy. In present ''*home-based interventional model*'' the only feasible diagnostic tool was sputum microscopy which was performed at nearest DMC on sputum specimens collected at home of subjects.

Saturday was the pre-fixed '*weekly contact evaluation day*' for TB-Unit Uri and Monday for TB-Unit Shopian. On these days the investigator first visited TU head quarter and after collecting list of due cases went to the home of the index cases registered during that week. Consent was obtained from the study subjects. Using modified WHO recommended sample format,[9] the investigator conducted detailed interview of index case and their household contacts available at home and evaluated them for P-TB symptoms and sputum specimens of symptomatic household contacts were collected for sputum microscopy.

Inclusion criteria was defined as bacteriologically confirmed index P-TB cases, *including transferred-in cases*, registered in the selected TB-Unit on DOTS from 1st January 2014 to 31st December 2014 and their household contacts of age ≥10 years as they were able to provide good quality sputum specimen when required.

Exclusion criteria was defined as those index P-TB cases and their contacts, who were not the permanent residents of the geographical area of selected TB Unit.

Although in proposed model, the duty of visiting homes of index cases has been fixed for the Medical Officer of concerned TB Unit but in present pilot study this job was done by the investigator himself.

2.1. Statistical analysis

The data was entered in ''Epi-Info 7'' software and analysed using ''Epi-Info 7'' and ''Open-Epi'' statistical soft wares. A p-value <0.05 was considered statistically significant.

3. Results

The *home-based interventional model* was applied to total 141 index cases and their total 598 eligible household contacts. The mean age of total 141 index cases was 42.0 ± 20.57 years where

Table 1 – Personal characteristics of household contacts influencing the acceptance and effectiveness of *home-based interventional model*.

Characteristics	Acceptance of evaluation by contacts (N = 598)			Case detection among household contacts (N = 598)		
	Participated [n = 423 (70.74%)]	Not participated [n = 175 (29.26%)]	Statistical significance	Detected [n = 27 (4.51%)]	Not detected [n = 571 (95.49%)]	Statistical significance
Gender						
Male	185 (56.23%)	144 (43.77%)	$\chi^2 = 74.33$;	14 (4.26%)	315 (95.74%)	$\chi^2 = 0.11$
Female	238 (88.48%)	31 (11.52%)	df = 1	13 (4.83%)	256 (95.17%)	df = 1
			$p < 0.0001$			$p = 0.7351$
Age group (years)						
10–20	150 (71.43%)	60 (28.57%)	$\chi^2 = 17.30$	10 (4.76%)	200 (95.24%)	$\chi^2 = 0.96$; df = 2
21–40	149 (64.78%)	81 (35.22%)	df = 3	12 (5.22%)	218 (94.78%)	$p = 0.6184$
41–60	89 (72.95%)	33 (27.05%)	$p = 0.0006$	5 (4.10%)*	117 (95.90%)	*Grouped together
>60	35 (97.22%)	1 (2.78%)		0*	36 (100%)	
Education						
Illiterate	145 (92.36%)	12 (7.64%)	$\chi^2 = 98.84$	9 (5.73%)*	148 (94.27%)	$\chi^2 = 3.108$
Literate and till 5th standard	65 (76.47%)	20 (23.53%)	df = 3	0*	85 (100%)	df = 2
6th to 10th standard	169 (70.71%)	70 (29.29%)	$p < 0.0001$	15 (6.28%)	224 (93.72%)	$p = 0.21140$
11th standard or above	44 (37.07%)	73 (62.39%)		3 (2.57%)	114 (97.43%)	*Grouped together
Occupation						
Home maker, Unemployed, Old age (>60 years)	202 (95.74%)	9 (4.26%)	$\chi^2 = 175$ df = 4 $p < 0.0001$	8 (3.80%)	203 (96.20%)	$\chi^2 = 0.44$ df = 2 $p = 0.7999$
Student	126 (72.41%)	48 (27.59%)		9 (5.17%)	165 (94.83%)	*All 'Outdoor workers' grouped together for the purpose of analysis
Agricultural, non-agricultural & Skilled workers	69 (63.30%)	40 (36.70%)		10 (9.18%)*	99 (90.82%)	
Business	12 (36.36%)	21 (63.64%)		0*	33 (100%)	
Govt./private service	14 (19.72%)	57 (80.28%)		0*	71 (100%)	

as the mean age of total 27 new cases detected among household contacts was found 27.8 ± 13.75 years. This difference between Index cases and co-prevalent cases among contacts was statistically significant (unpaired *t*-test; $p = 0.0004$).

Table 1 *reveals the influence of personal characteristics of household contacts- gender, age group, education and occupation on the acceptance and effectiveness of intervention*. The effect of gender ($p < 0.0001$), age group ($p = 0.0006$), education ($p < 0.0001$), occupation ($p < 0.0001$) of household contacts on *acceptance of interventional model* was statistically significant whereas association of gender ($p = 0.73510$), age group ($p = 0.61840$), education ($p = 0.21140$), occupation ($p = 0.79990$) of household contacts on *effectiveness of interventional model* in terms of detection of co-prevalent and secondary incidental cases of pulmonary tuberculosis among household contacts was statistically not significant.

Table 2 *shows the effect of relationship of household contact with Index case on acceptance and the yield of intervention. The relationship of household contacts with index case* ($p < 0.0001$) was statistically significantly affecting the acceptance of interventional model. The *relationship of household contact with index P-TB Case* was also statistically *significantly* ($p = 0.04188$) influencing the detection of *co-prevalent and incident secondary cases* of sputum smear positive P-TB among themselves. Overall active P-TB was most common (9.73%) among siblings.

Table 3 *shows family level characteristics on effectiveness of intervention for active case finding among household contacts*. Out of family level characteristics only *overcrowding in house* ($p = 0.0017$) was found to statistically significantly influencing the effectiveness of interventional model and such other determinants including type of family ($p = 0.90860$), type of house ($p = 0.12870$), ventilation in the house ($p = 0.56410$), socioeconomic class (*upper*, *middle* and *lower*) of family ($p = 0.10340$) and category (general or scheduled tribe) of subjects ($p = 0.91560$) were not found statistically significantly affecting the yield of interventional model.

Table 4 *reveals effect of characteristics of index cases on detection of co-prevalent cases of pulmonary TB in the family by the application of home-based interventional model*. The deference between detection of P-TB cases among household contacts living with specific ''type'' of index P-TB Cases (new or recurrent) was statistically not significant ($p = 0.32760$).

The deference between detection of P-TB cases among household contacts living with index P-TB cases of specific ''bacterial load'' (grade of sputum smear positivity for AFB: Scanty, 1+, 2+, or 3+) was statistically not significant ($p = 0.55950$).

Out of 424 household contacts of ''New'' index cases, whose status at 6th month was found ''cured'' (*proxy indicator for compliance of index cases to DOTS*), 4.43% household contacts were detected as sputum smear positive cases while out of total 174 household contacts living with *group of index cases* whose status was ''other than cured'' at 6th monthly follow up timing in present study, (*including 'relapse' index cases on Cat. II DOTS*), 5.17% household contacts were detected as sputum

Table 2 – Effect of "relationship between household contact and index case" on acceptance and effectiveness of *home-based interventional model*.

Relationship of household contact with index case	Acceptance of evaluation by contacts (N = 598)			Case detection among household contacts (N = 598)		
	Participated [n = 423 (70.74%)]	Not participated [n = 175 (29.26%)]	Statistical significance	Detected [n = 27 (4.51%)]	Not detected [n = 571 (95.49%)]	Statistical significance
Spouse (husband or wife)	67 (89.33%)	8 (10.67%)	$\chi^2 = 44.85$ df = 6	2 (2.67%)	73 (97.33%)	$\chi^2 = 9.91$ df = 4
Parent (father or mother)	64 (69.56%)	28 (30.44%)	$p < 0.0001$	2 (2.17%)	90 (97.83%)	$p = 0.0418$ *Grouped
Siblings (brother or sister)	68 (60.17%)	45 (39.83%)		11 (9.73%)	102 (90.27%)	together for the purpose
Children (son or daughter)	106 (60.91%)	68 (39.09)		8 (4.60%)*	166 (95.40%)	of analysis
Daughter-in-law	49 (96.08%)	2 (3.92%)		1 (1.96%)*	50 (98.04%)	
Grand-child	32 (68.09%)	15 (31.91%)		0*	47 (100%)	
Other relationship[a]	37 (80.44%)	9 (19.56%)		3 (6.52%)*	43 (93.48%)	

[a] The complete list of "other relationship" include: (a) Other in-laws – (i) father-in-law (5); (ii) mother-in-law (15); (iii) brother-in-law (9); (iv) sister-in-law (23); (v) grand-daughter-in-law (3); (vi) son-in-law (3). (b) Others – (i) Grand-parent (7); (ii) uncle (2); (iii) aunt (3); (iv) cousin (3); (v) nephew (4); (vi) niece (2); (vii) servant (2).

Table 3 – Effect of "characteristics of family of index case" on effectiveness of *home-based interventional model*.

Characteristics	Case detection among contacts evaluated (N = 598)			Characteristics	Case detection among household contacts (N = 598)		
	Detected [n = 27 (4.51%)]	Not detected [n = 571 (95.49%)]	Statistical significance		Detected [n = 27 (4.51%)]	Not detected [n = 571 (95.49%)]	Statistical significance
Type of family				*Type of house*			
Nuclear	16 (4.60%)	332 (95.40%)	$\chi^2 = 0.01$	Pacca	19 (3.88%)	479 (96.12%)	Fisher exact.
Joint	11 (4.40%)	239 (95.60%)	df = 1	Semi-pacca	8 (8.00%)	92 (92.00%)	$p = 0.1287$
			$p = 0.9086$				
Over-crowding in house				*Ventilation in house*			
Absent	12 (3.06%)	413 (96.94%)	$\chi^2 = 9.75$	Adequate	23 (4.28%)	515 (95.72%)	Fisher exact
Present	15 (8.67%)	158 (91.33%)	df = 1	Inadequate	4 (6.67%)	56 (93.33%)	$p = 0.5641$
			$p = 0.0017$				
Socioeconomic class[a]				*Social category*			
Upper	3 (5.56%)	51 (94.44%)	$\chi^2 = 4.53$	General	21 (4.47%)	449 (95.53%)	$\chi^2 = 0.01$
Middle	13 (26.30%)	311 (73.70%)	df = 2	Scheduled Tribe	6 (4.69%)	122 (95.31%)	df = 1
Lower	11 (9.17%)	109 (90.83%)	$p = 0.1034$				$p = 0.9156$

[a] BG Prasad's Socioeconomic Scale (Revised – January 2014): upper (1st), middle (2nd and 3rd), lower (4th and 5th).

smear positive P-TB Cases during the intervention of active case finding. Therefore, the "status of index P-TB Case at 6th month" has statistically no significant ($p = 0.43860$) influence on detection of P-TB among household contacts.

Table 5 *shows prevalence of tuberculosis related risk factors among household contacts and their effect on effectiveness of intervention under study*. In present study, the presence of established "lifestyle risk factors for TB" (*smoking*, *alcoholism*, and *drug abuse*) among household contacts has statistically no significant ($p = 0.25550$) association with detection of co-prevalent or incidental secondary active P-TB cases among themselves. Out of total 4 household contacts with past history of TB, none was found as relapse case of TB during the intervention in present study while out of total 419 household contacts *without any past history of TB*, 5.01% were detected as sputum smear positive cases during home-based intervention. Similarly, the presence of known "chronic diseases associated with TB" (*asthma*, *diabetes mellitus*, *renal failure*, *malignancy*) among household contacts has statistically no significant ($p = 0.19860$) impact on detection of sputum smear positive P-TB among themselves.

Table 6 *shows the association between the level of exposure of household contacts on effectiveness of model in terms of co-prevalent P-TB case detection*. The "*Average daily time period*" for which any household contact lives with an active case of P-TB has statistically no significant ($p = 0.91300$) influence on detection of sputum smear positive P-TB cases among themselves. The "period" (in years) during which the household contacts were living in same house as that of Index P-TB *Case (applicable for 'In-law' relationship and servants)* has statistically no significant

Table 4 – Effect of "characteristics of Index cases (N = 141)" on effectiveness of *home-based interventional model*.

Characteristics	Case detection among household contacts (N = 598)		
	Detected [n = 27 (4.51%)]	Not detected [n = 571 (95.49%)]	Statistical significance
Type of index cases			
New	25 (4.58%)	521 (95.42%)	Fisher exact.
Relapse	2 (3.85%)	50 (96.15%)	$p = 0.3276$
Grade of positivity of index case			
*Scanty	6 (8.96%)	61 (91.04%)	$\chi^2 = 1.161$
*1+	9 (2.92%)	299 (97.08%)	df = 2
2+	7 (4.67%)	143 (95.33%)	$p = 0.5595$
3+	5 (6.85%)	68 (93.15%)	*Grouped together
Status of index case at 6th month			
Cured (n = 100)	18 (4.43%)	406 (95.57%)	$\chi^2 = 0.59$
Treatment completed (n = 19)	3 (3.33%)*	78 (96.23%)	df = 1
Continued on CP[a] (n = 18)	4 (5.56%)*	68 (94.44%)	$p = 0.4386$
Shifted to Cat IV (n = 1)	1 (25.00%)*	3 (75.00%)	*Grouped together for the purpose of analysis
Pt died while on DOTS (n = 2)	0*	10 (100%)	
Shifted to non-DOTS by PP[b] (n = 1)	1 (14.29%)*	6 (85.71%)	

[a] Includes index cases on Cat. II treatment and index cases where "Intensive phase" was extended.
[b] "Shifted to non-DOTS by Private Practitioner": this term is not official under RNTCP but this is a finding in Kashmir.

Table 5 – Effect of presence of "risk factors" among household contacts of Index case on effectiveness of *home-based interventional model*.

Risk factors		Case detection among household contacts evaluated (N = 423)		
		Detected [n = 27 (6.38%)]	Not detected [n = 396 (93.62%)]	Statistical significance
Life style risk factors {smoking; alcoholism[a]; drug abuse[a]}	Present	9 (1.25%)	63 (98.75%)	Fisher exact
	Absent	18 (5.12%)	333 (94.88%)	$p = 0.0510$
Past history of tuberculosis	Present	1 (1.42%)	6 (98.58%)	Fisher exact
	Absent	26 (6.25%)	390 (93.75%)	$p = 0.7439$
History of specific chronic diseases {*asthma, diabetes mellitus*; renal failure[a], malignancy[a]}	Present	5 (11.62%)	38 (88.38%)	Fisher exact
	Absent	22 (5.78%)	358 (94.22%)	$p = 0.2529$

[a] Although subjects were asked about these risk factors/condition but no such household contact was found.

Table 6 – Effect of "Level of exposer" of household contacts to index case on effectiveness of *home-based interventional model*.

Level of exposer		Case detection among household contacts evaluated		
		Detected	Not detected	Statistical Significance
Average daily time period of living in '*close contact*'	≤12 h	18 (5.52%)	308 (94.48%)	$\chi^2 = 1.76$
	>12 h	9 (9.28%)	88 (90.72%)	df = 1
	Total	*27 (6.38%)*	*396 (93.62%)*	$p = 0.1841$
Period of living in the same house[a]	≤10 years	1 (0.25%)	39 (99.75%)	Fisher exact
	>10 years	2 (6.89%)	29 (93.11%)	$p = 0.8082$
	Total	*3 (4.22%)*	*68 (95.78%)*	
Bed sharing with index case[b]	Yes	7 (7.44%)	87 (92.56%)	Fisher exact
	No	4 (6.25%)	60 (93.75%)	$p > 0.99999$
	Total	*11 (6.96%0*	*147 (93.04%)*	

[a] Applicable only for relationship of 'In-laws' or 'servant' *between household contact and index case.*
[b] Applicable only when index case and/or HHC are of (i) same gender and age <20 years or (ii) spouses.

($p = 0.37420$) influence on detection of active P-TB cases among those household contacts. The "*bed sharing practice*" between index P-TB and their household contacts (which is common among siblings during winter in Kashmir) has statistically no significant ($p > 0.99999$) influence on detection of active P-TB cases among household contacts.

4. Discussion

Active case finding among household contacts of passively detected index case of pulmonary tuberculosis is an effective intervention for tuberculosis programme. The programme

managers have many apprehensions regarding various factors adversely affecting the outcome of *intervention of active case finding* among household contacts of index pulmonary tuberculosis cases. Present study deals with study of such factors. Many issues regarding the acceptance of Home-based Intervention could be solved by fixing weekly contact evaluation day and prior information to the family about the purpose and schedule of initial home visit by Medical Officer as described in the design model of intervention. In present study all eligible household contacts present at home during the home visit by the investigator agreed to participate for his/her evaluation for symptoms of tuberculosis and all symptomatic subjects provided sputum sample for microscopy. Overall participation of eligible household contacts was 70.74%. Other 29.26% household contacts were absent from home at the time of visit by the investigator due to their personal reasons.

The mean age of new cases detected among household contacts was statistically significantly lower than the mean age of passively detected pulmonary tuberculosis cases. This shows that by active case finding we may able to diagnose pulmonary tuberculosis among younger subjects who are otherwise suffering from relatively mild symptoms of P-TB and with no co-morbidity therefore remain un-diagnosed in the community.

During the years 2002–2004 in Iran, Khalilzadeh et al.,[10] conducted a cross sectional study to assess the impact of contact screening on case-finding. In that study out of total 227 household contacts of 68 index cases, 17 (20.2%) contacts were found positive for acid fast bacilli. None among father, mother or spouse of index cases were found positive for AFB. Out of these 17 new cases, 6 (7.1%) were having relationship of son/daughter with the index case, and 11 (13.0%) were having other relationship with index case. Thus the impact of relationship on case detection was found statistically significant ($p = 0.005$). Out of same 17 cases among contacts, 3 (17.6%), 7 (41.2%) and 7 (41.2%) were exposed to index cases with their grade of sputum positivity as one plus, two plus and three plus respectively. Thus the effect of grade of sputum smear positivity was not statistically associated with detection of cases with active TB among contacts ($p = 0.8$).

Present study which deals with factors affecting applicability of interventional model. In this study the sample size to find out the association between factors/determinants and acceptance of the interventional model was adequate but among of total 598 household contacts only 27 active pulmonary TB were detected. Therefore, on application of relevant statistical tests of significance on small number (sample) of these 27 *co-prevalent and co-incident secondary pulmonary TB cases*, it was difficult to draw appropriate conclusion about the results. This was the limitation of present study.

The tuberculosis control programme managers should have evidence based effective interventions to implement for the success of programme. Although investigator of present pilot study has collected, collated and analysed data regarding factors affecting the applicability of proposed *home-based interventional model*, it is only for academic interest and it has no operational applicability in RNTCP. Therefore, it is not necessary for every Medical Officer of TB-Units to go for such minute details while practicing the proposed *home-based interventional model* for active case finding among the household contacts. They can limit their task to symptomatic screening of household contacts, followed by collection of sputum specimen for microscopy. In future, when this proposed *interventional model* get its endorsement under RNTCP and implemented in majority of TB-Units, then another large scale study can be done regarding factors/determinants of interest associated with outcome of intervention.

5. Conclusion

Present study was conducted to address the apprehensions of tuberculosis control programme managers regarding the various factors or determinates which could affect the acceptance and effectiveness of proposed *home-based interventional model for contact tracing*. Despite variability of characteristics, the overall acceptance of intervention was found 70.74%. The association between personal characteristics of household contacts and acceptability of interventional model was evident. Most of the factors (except ''*overcrowding in house*'' and ''*relationship of household contact with the index case*'') have statistically insignificant influence on effectiveness of the *home-based interventional model* for active case finding among household contacts.

Conflicts of interest

The authors have none to declare.

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Original Article

Endobronchial ultrasound experience in a high tuberculosis prevalence setting

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ABSTRACT

Background: Most of the published endobronchial ultrasound-guided transbronchial needle aspiration (EBUS–TBNA) data are from the western countries, establishing the role of EBUS–TBNA in the diagnosis and staging of lung cancer. The etiology of mediastinal lymphadenopathy may be different in an ethnic group with a high prevalence of tuberculosis (TB).
Objective: To assess the etiology of mediastinal adenopathy in a high TB prevalence setting and to determine the performance of various tests in the diagnosis of tuberculous mediastinal lymphadenitis.
Methods: Retrospective analysis of bronchoscopic data of patients who underwent endobronchial ultrasound (EBUS) in a tertiary care center in India.
Results: Out of 138 patients who underwent EBUS, 63 (46%) had granulomatous disease. Of the 35 patients with a diagnosis of TB, in 10 (29%), microbiology of EBUS specimens was diagnostic and in 3 (9%), this was the sole diagnostic feature. In 5 (14%) mycobacterial cultures were positive, in 6 (17%) GeneXpert for Mycobacterium tuberculosis/rifampicin resistance (Xpert MTB/RIF) was positive, and in 3 (9%) acid fast smears were positive.
Conclusion: In high TB prevalence countries, EBUS diagnoses a higher number of granulomatous than malignant diseases. EBUS specimen should, therefore, be subjected also to mycobacterial smear, culture, and Xpert MTB/RIF for optimal results.

1. Introduction

Endobronchial ultrasound (EBUS) allows visualization of mediastinal structures and convex probe EBUS allows real time sampling of the mediastinal and hilar lymph nodes. Most of the published data on endobronchial ultrasound guided transbronchial needle aspiration (EBUS–TBNA) are from the western countries. These data have established the role of EBUS–TBNA in the diagnosis and staging of lung cancer. It is likely that the etiology of mediastinal lymphadenitis would be different in a different ethnic group, particularly in those with a

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Abbreviations: EBUS, endobronchial ultrasound; TBNA, transbronchial needle aspiration; Xpert MTB/RIF, GeneXpert for Mycobacterium tuberculosis/rifampicin resistance; TB, tuberculosis; CT, computer tomography; AFB, acid fast bacilli.

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Table 1 – Spectrum of diagnosis in patients who underwent EBUS.

Diagnosis		n = 138 (%)
Granulomatous disease n = 63 (46%)	Tuberculosis	27 (20)
	Sarcoidosis	27 (20)
	Tuberculosis and sarcoidosis	8 (6)
	Sarcoidosis and malignancy	1 (1)
Others n = 75 (54%)	Malignancy	43 (31)
	Non-diagnostic	29 (21)
	Non-representative	3 (2)

EBUS – endobronchial ultrasound.

high prevalence of tuberculosis (TB). In this group, until the recent advent of endoscopic ultrasound (EUS) Fine needle aspiration (FNA)/EBUS–TBNA, since mediastinoscopy service was either not easily accessible or too invasive for these patients, those with mediastinal adenopathy alone often received a therapeutic trial with antitubercular drugs. Also, differentiating sarcoidosis from TB is a challenge, since both these conditions would reveal granulomatous inflammation, and a clinico-radiological correlation would often be needed. Although presence of caseation is in favor of TB, not all TB lymphadenitis would reveal this finding. Furthermore, microbiological studies including Mycobacterial culture could turn out to be negative, fairly commonly.[1] EBUS adds to the armamentarium to clinch the diagnosis of mediastinal lymphadenitis. We report our experience of 138 consecutive patients who underwent EBUS–TBNA.

2. Methods

We performed a retrospective analysis of clinical, radiologic, and bronchoscopic data of patients who underwent EBUS in a tertiary care center in southern India from May 2011 to December 2013. Institutional ethics committee approved the study. Written informed consent had been obtained from the subjects for the procedure, as per institutional protocol. All patients had a computed tomography (CT) scan confirming mediastinal adenopathy.

It was departmental policy to carefully search for more accessible nodes before proceeding to EBUS. Therefore, it could be said that anyone who underwent EBUS–TBNA had no other more accessible node for biopsy. Likewise, all patients with chest X-ray opacities and sputum production were subjected initially to sputum microscopy to exclude TB, before EBUS was performed.

EBUS was done as a day care procedure with conscious sedation using midazolam and fentanyl as per standard protocol. EBUS scope (BF_UC180F; Olympus Medical Systems, Singapore) with the compatible endoscopic unit was used. Under real time guidance, the lesions were punctured with disposable 21-gauge Vizishot needle (NA-201SX-4021 Olympus Medical Systems, Singapore). The needle was moved back and forth with or without suction. Depending on the samples obtained, up to 4 passes were made in to each node. The sample was considered as representative if it showed a preponderance of lymphocytes or pathological cells. As clinically indicated, bronchoalveolar lavage (BAL), endobronchial biopsy and transbronchial lung biopsy (TBLB) were also done. EBUS and bronchoscopic specimens were apportioned for testing based on the quantity obtained, and the likelihood of the tests contributing to the diagnosis.

The EBUS sample was normally sent for cytology, polymerase chain reaction testing for TB (GeneXpert for Mycobacterium tuberculosis/rifampicin resistance – Xpert MTB/RIF) and mycobacterial cultures. Rapid onsite evaluation (ROSE) was not performed in most cases. The results of these tests contributed to the final diagnosis, along with the clinico-radiologic features.

3. Results

Out of 138 patients who underwent EBUS, it was diagnostic in 106 (77%) – 63 (46%) had granulomatous disease and 43 (31%) malignancy. EBUS was non-diagnostic in 29 (21%) lymph node samples. The sample was not representative of lymph node in 3 (2%).

Out of 63 patients with granulomatous disease, 27 (43%) were ultimately diagnosed as sarcoidosis, 27 (43%) as TB, 8 (13%) as both sarcoidosis and TB, and 1 (1.6%) as both malignancy and sarcoidosis (Table 1). The baseline characteristics of the patients with granulomatous disease are tabulated in Table 2. Subcarinal and right lower paratracheal lymph nodes were the most common groups of lymph nodes that were involved in this group.

Of the 35 patients with a diagnosis of TB, in 10 (29%), microbiology of EBUS specimens was diagnostic, and in 3 (9%), this was the sole diagnostic feature. In 5 (14%) mycobacterial cultures were positive, in 6 (17%) Xpert was positive, and in 3 (9%) AFB smears were positive. None of them samples tested

Table 2 – Baseline characteristics of patients with granulomatous disease on endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) (n = 63).

Characteristic	
Average age – stratified by diagnosis	
Tuberculosis (27)	37
Sarcoid (27)	48
Tuberculosis and sarcoid (8)	43
Malignancy with sarcoid (1)	42
All (63)	42
Sex	
Male	23 (37%)
Female	40 (63%)
Lymph node station and size on CT[a] (n = 62)	(short axis on CT in mm, range)
Subcarinal 7 (n = 44)	22.3 (6–45)
Right lower paratracheal 4R (n = 51)	23.3 (7–45)
Left lower paratracheal 4L (n = 3)	22
Right upper paratracheal 2R (n = 1)	30
Right hilar 10R (n = 5)	12.6 (8–16)
Left hilar 10L (n = 2)	22.5
Left interlobar 11L (n = 1)	26

[a] 1 patient had a CT film without a scale and hence lymph node dimensions could not be measured.

Table 3 – Performance of individual tests among the 35 patients diagnosed as tuberculosis.

Test	No. of patients in whom performed	No. of patients in whom positive	Yield in %
AFB smear of TBNA samples	27	3	11%
Mycobacterial culture of TBNA samples	22	5	23%
Xpert of TBNA samples	10	6	60%
TBNA cytology	35	26	74%
TBLB histology	20	14	70%

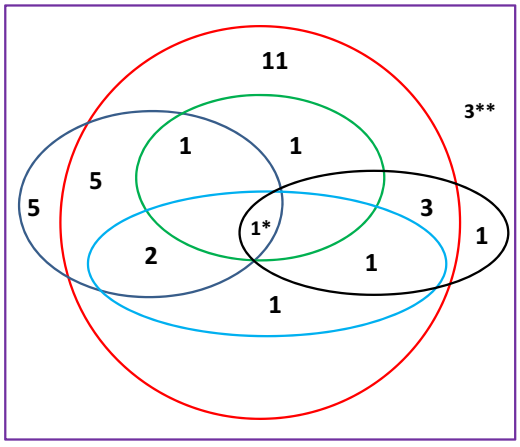
AFB – acid fast bacilli; TBNA – transbronchial needle aspiration; TBLB – transbronchial lung biopsy.

were rifampicin resistant on Xpert. In these patients who were diagnosed as TB, cytological studies of TBNA samples provided the highest yield. Among the microbiological tests performed on the TBNA samples, Xpert MTB/RIF appears to provide the highest yield (Table 3). The diagnostic overlap in this category is depicted in Fig. 1.

4. Discussion

In countries with high TB prevalence, granulomatous mediastinal lymphadenopathy is common and often it is challenging to differentiate TB and sarcoidosis. The clinico-radiological picture and cytological pictures may overlap[2,3] Caseation may favor TB, but it is not always present. AFB smear test is often negative. Although mycobacterial culture is the gold standard for TB diagnosis, it has low sensitivity.

In a study of peripheral tubercular lymphadenopathy from India, overall smear positivity among TB lymph nodes was 49.4%.[1] The culture positivity varied from 50% in non-necrotic granulomatous lesions to 83.3% in necrotic lesions. More importantly, in 28 of the161 TB lymph nodes cases, when the cytology was non-contributory, mycobacterial culture was positive, emphasizing the need for incorporating culture as a



*Positive for AFB smear, Mycobacterial culture, Xpert and TBLB as depicted, **but was negative for cytology**.

**The 3 patients outside are those who had only a clinical diagnosis and improved on anti-tuberculous treatment.

Fig. 1 – Diagnostic overlap among tuberculosis patients (total = 35).

Table 4 – Comparison with similar studies:.

	Total	Non-representative	Granulomatous disease	sarcoidosis	Tuberculosis	EBUS TBNA AFB+	EBUS TBNA MTB culture+
Srinivasan et al. (2013)	39	2	21 (54%)	12 (57%)	9 (43%)	2	4
Kaur (2013)	49					8	5
Madan et al. (2014)	102	4	62 (61%)	34 (55%)	28 (45%)	16	Not available
This study	138	3	63 (46%)	28 (44%)	27 (43%)	3	5
				Both 8 (13%)			

EBUS–TBNA – endobronchial ultrasound transbronchial needle aspiration; AFB – acid fast bacilli; MTB – Mycobacterium tuberculosis.

routine laboratory investigation in all cases of suspected tuberculous lymphadenitis.

With the introduction of EUS and EBUS, the mediastinal lymph nodes have also became more accessible for evaluation. In another series from India, Manucha et al. report 269 patients with mediastinal lymphadenopathy diagnosed by EUS, and cytological diagnosis of granulomatous lymphadenitis was obtained in 206 cases.[4] Of these, TB could be established as an etiology in 76 cases only on the basis of AFB positivity or necrosis. Sarcoidosis was diagnosed in 7 cases and in the remaining 123 cases, the etiology of granulomatous lymphadenitis could not be established and clinical correlation was suggested. This study highlights the diagnostic dilemma in distinguishing TB from sarcoidosis in regions with high prevalence of TB.

Kaur et al. evaluated 49 cases of granulomatous lymphadenitis diagnosed by EBUS.[5] In 14 cases, caseation was observed. AFB staining by Zeil Neelson was positive in 8 of the 13 cases in which it was performed. Culture for mycobacteria was positive in only 3 cases among 10 cases in which it was done. Culture of EBUS specimen was done in 26 cases of non-caseating granulomatous lymph nodes and was positive only in 2 cases. Few small loose granulomas are usually observed in TB, compared to numerous compact granulomas in sarcoidosis. We attempted to classify non-caseating granulomatous inflammation into TB and sarcoidosis based on cytologic features. However, this observation could not be substantiated in the present study. In our series, in most cases, it was not possible to categorically distinguish TB and sarcoid granulomas by cytology. Although EBUS is a novel non-invasive technique for diagnosing granulomatous disease, the precise identification of the etiology may still require the correlation of cytologic, microbiologic, clinical, and radiological data in TB endemic regions.

Navani et al., in a multicentric study from the United Kingdom, reported that with the use of EBUS–TBNA, a diagnosis of TB could be established in 146 of the 156 patients with intrathoracic tubercular lymphadenitis.[6] Smears were positive for AFB in 27 cases while in 74 cases Mycobacterium tuberculosis grew on culture. Smears were positive with negative culture in 8 cases. Thus, microbiologic confirmation was obtained in 82 of the 146 cases.

In a recent study from India similar to ours, Madan et al. have analyzed their EBUS data on 102 patients, which revealed TB in 28 of them and sarcoidosis in 38 of them.[7] Higher number of the TB specimens had AFB smear positivity (16 of 28) and Xpert was the sole diagnostic modality in 4 patients. The comparison of results of the above mentioned studies and our study in diagnosing granulomatous diseases has been tabulated in Table 4.

5. Conclusion

In high TB prevalence countries, EBUS diagnoses a higher number of granulomatous than malignant diseases. EBUS specimen should be subjected to mycobacterial smear, culture, and Xpert MTB/RIF for optimal results. Furthermore, Xpert MTB/RIF may help in not only providing rapid diagnosis, but also information on resistance to rifampicin, and thus the diagnosis of multidrug-resistant TB.

Conflicts of interest

The authors have none to declare.

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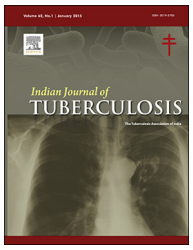

Original Article

The success and safety profile of sputum induction in patients with chronic obstructive pulmonary disease: An Indian experience

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ABSTRACT

Background: Neutrophilic inflammation is common in chronic obstructive pulmonary disease while Asthma COPD overlap syndrome has eosinophilic predominance. Identifying the type of inflammation will aid in better management of COPD, but published studies show that induced sputum examination is more frequently used in asthma than COPD, with safety being the limiting factor. We aimed to determine the success and safety of sputum induction (SI) in COPD patients.

Methods: 116 stable COPD patients underwent SI. Success was defined as adequate sputum sample resulting in a cytospin sufficient to assess differential count while safety by the fall in FEV_1.

Results: The mean (SD) FEV_1% predicted post bronchodilator was 58.8 (17.8) and 59 (51.8%) patients had moderate COPD. Success was 98.28%. The procedure was safe with overall fall in FEV_1 of 11.1% (5.1, 15.2). ≥20% fall was noted in 13 (11.4%) patients, 10–20% in 24 (21.0%) patients, and less than 10% in 29 (25.4%) patients while 48 (42.1%) had no fall. There was an inverse correlation between reversibility in FEV_1 and percentage fall in FEV1; $r = -0.437$ and $p = 0.001$. Stepwise multivariate linear regression showed reversibility as an independent predictor of fall in FEV_1; $R^2 = 0.137$.

Conclusions: Sputum induction is successful and safe in COPD. Even a fall in $FEV_1 > 20\%$ is reversible.

1. Introduction

Airway inflammation is fundamental to the pathogenesis of chronic obstructive pulmonary disease (COPD). The neutrophils are the main inflammatory cells that secrete a variety of proinflammatory chemokines and cytokines.[1] The presence and the type of inflammation can be detected on bronchial washings, bronchial biopsy, or bronchoalveolar lavage, but bronchoscopy is invasive, expensive, and is associated with

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complications. Induced sputum is a relatively noninvasive and reliable procedure, and the quantitative cell counts in induced sputum reflect the type of inflammation.[2]

Induced sputum analysis is being used in some countries in the diagnosis and management of obstructive airway diseases like chronic obstructive airway disease, asthma, and bronchiectasis,[3,4] while Spanish, Finish, Czech guidelines suggest use of induced sputum in the diagnosis and management of asthma COPD overlap syndrome (ACOS).[5–7] Eosinophilic inflammation in the airways of smokers responds to steroids[8] unlike noneosinophilic inflammation.[9] However, induced sputum studies are not widely used in our country; one of the factors limiting its use may be its safety in COPD.[10] Therefore, we aimed to assess the success and safety of sputum induction in stable COPD patients.

2. Methods

2.1. Study setting, design, and study population

This was a prospective, observational study conducted at a tertiary care teaching hospital. The study was approved by the Institutional Ethics Committee, and all participants gave informed consent. A total of 116 patients ≥40 years of age having chronic obstructive pulmonary disease were enrolled. COPD was diagnosed by GOLD guidelines with a postbronchodilator $FEV_1/FVC < 70\%$ in a stable clinical state.[11] We excluded patients with acute exacerbation of COPD within 4 weeks of enrollment, use of systemic steroids within six weeks of enrollment, and COPD patients with any other respiratory disease.

Spirometry was performed as per ATS/ERS 2005 guidelines.[12] Respiratory symptoms and risk factors for COPD were evaluated using the BOLD Core and BOLD biomass and fuel questionnaire.[13] Dyspnea was assessed using the modified Medical Research Council (mMRC) dyspnea scale.[14]

2.2. Sputum induction and processing

Sputum was induced by the method described by Pizzichini et al.[15] Trained personnel used standard operating procedures while performing sputum induction. FEV_1 values were measured before bronchodilation and the highest FEV_1 was recorded as baseline FEV_1. Fifteen minutes after inhalation of 200 mcg of salbutamol through metered dose inhaler, postbronchodilator spirometry was performed. Sputum induction was performed as per postbronchodilator FEV_1. If FEV_1 post bronchodilator was <800 ml, the subject was nebulized with normal saline (0.9%), otherwise hypertonic saline in increasing concentration (3%, 4%, 5%) was used. Nebulization was carried out with ultrasonic nebulizer (Smart Care) with an output set to 0.6–0.8 ml/min. Subjects inhaled saline for five minutes and were informed to report symptoms of breathlessness or chest tightness. After saline inhalation, the subject was asked to rinse the mouth and throat with water and to cough and expectorate in a sterile container. The procedure was stopped when the selected sputum obtained was greater than 0.8 g.

Postsaline inhalation, spirometry was performed to assess safety of the procedure. If the fall in FEV_1 was ≥20% from the postbronchodilator value, then the induction process was stopped and bronchoconstriction reversed with inhaled salbutamol. Excessive bronchoconstriction was defined as fall in FEV_1 of ≥20%. If FEV_1 drop was <10% and sputum obtained was inadequate, then the subject was nebulized with the next concentration of hypertonic saline for another five minutes. Prior to discharge from site, patient's FEV_1 was measured, and it was considered safe to discharge when FEV_1 was within 90% of baseline FEV_1 (prebronchodilator level).

Sputum processing was done on ice as per standardized methodology of sputum induction and processing.[3,16] The selected sputum was mixed with 0.1% DTT (dithiothreitol) in the ratio 1:4 and vortexed for 15 s, and then put on a bench rocker and rocked for 15 min. To stop the effect of DTT, four times volume of DPBS (Dulbecco's phosphate buffer saline) was added, filtered, and total cell count obtained using trypan blue exclusion method. The remaining cell suspension was centrifuged at $790 \times g$. The cell pellet was resuspended in DPBS to 1×10^6 cells per ml and cytospun. Slides were stained for differential cell counts. Sputum was processed within two hours of collection. Success of sputum induction was defined when an adequate sputum sample provided a cytospin with appropriate differential count.

2.3. Statistical analysis

Statistical analysis was performed using IBM Statistical Package for Social Sciences (SPSS) version 23. Variables are described as mean and standard deviation (SD) for normally distributed continuous data and median and interquartile range (IQR) for nonparametric data. Categorical variables are presented as percentages. Independent Student's *t* test, Chi-square test, and a paired *t* test were applied to study the differences between groups and repeated measures, respectively. One-way ANOVA was applied to study lung function during sputum induction between severity grades of COPD. Correlation was analyzed by Pearson's and Spearman's rank correlation test, and a multivariable linear regression was applied to predict the fall in FEV_1 following sputum induction. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Subject characteristics

Of the 116 patients, 114 gave adequate sample. In the sputum safety analysis group, there were 68 males and 46 females; mean (SD) age was 64.33 (7.9) years. Prior to sputum induction, wheeze was the most common symptom (71.9%), and commonest grade of dyspnea was mMRC Grade 2. The mean (SD) FEV_1% predicted post bronchodilator was 58.8 (17.8) and males had significantly lower FEV_1% predicted than females; [55.6 (16.5), 63.6 (18.8)] $p = 0.01$. Majority of patients had moderate COPD, i.e., 59 (51.8%) patients. See Table 1 for baseline patient characteristics.

Table 1 – Baseline characteristics of COPD patients.

	Whole group	Males	Females	*p* value
n	114	68 (59.6)	46 (40.4)	0.116
Age	64.33 ± 7.9	65.63 ± 7.5	62.41 ± 8.1	0.03*
Symptoms				
Cough	23 (20.2)	17 (25)	6 (13)	0.11
Sputum	50 (43.9)	34 (50)	16 (34.8)	0.10
Wheeze	82 (71.9)	49 (72.1)	33 (71.7)	0.97
Spirometry				
FEV_1 ml post BD	1138.6 ± 428.3	1286.5 ± 452.3	920 ± 272.3	0.001*
FEV_1% post BD	58.8 ± 17.8	55.6 ± 16.5	63.6 ± 18.8	0.01*
FEV_1/FVC % post BD	54.2 ± 9.5	52.3 ± 9.5	57.0 ± 9.0	0.009*
Severity of disease				
Mild	16 (14)	8 (11.8)	8 (17.4)	0.21
Moderate	59 (51.8)	32 (47.1)	27 (58.7)	
Severe	37 (32.5)	26 (38.2)	11 (23.9)	
Very severe	2 (1.8)	2 (2.9)	0	
mMRC grades				
0	6 (5.3)	3 (4.4)	3 (6.5)	0.69
1	26 (22.8)	18 (26.5)	8 (17.4)	
2	72 (63.2)	41 (60.3)	31 (67.4)	
3	10 (8.8)	6 (8.8)	4 (8.7)	

Data are presented as mean ± SD; data in parentheses are presented as *n* (%). FEV_1 = forced expiratory volume in one second, FVC = forced vital capacity, BD = bronchodilator, mMRC = modified Medical Research Council.
* Statistically significant.

3.2. *Success of sputum induction*

Of the total 116 stable COPD patients, two failed to give adequate sputum sample. The remaining patients' sputum cytospin slides showed adequate viability and appropriate differential count. Thus, the overall success was 98.28%.

3.3. *Safety of sputum induction*

The procedure was well tolerated and no patient complained of breathlessness, wheezes, or chest discomfort during sputum induction process.

The mean (SD) of FEV_1 pre- and postbronchodilator on the day of sputum induction was 1057.6 (407.2) ml and 1169.8 (430.9) ml, respectively, while postsaline FEV_1 was 1123.1 (444.7) ml. Prebronchodilator FEV_1 improved significantly after salbutamol (mean difference was −118 ml, $p = 0.001$), while mean difference between postbronchodilator and postinduction was 46 ml, $p = 0.00$. 18 (15.7%) subjects had $FEV_1 < 800$ ml and hence received 0.9% saline nebulization, while the rest gave adequate sputum with 3% hypertonic saline. The procedure was safe with overall fall in FEV_1 post-SI of 11.1% (5.1, 15.2), minimum fall of 1.8%, and maximum of 33.0%. More than 20% fall was noted in 13 (11.4%) patients, 10–20% in 24 (21.0%) patients, and less than 10% in 29 (25.4%) patients, while 48 (42.1%) patients had no fall. The subjects who received normal saline, i.e., 4 (22.2%) patients, had a more than 20% drop compared to 9 (9.37%) patients who received hypertonic saline nebulization, $p = 0.116$, see Fig. 1. As female patients had a statistically lower FEV_1 in ml than males, we compared the percentage fall in FEV_1 in both genders and found no difference (Table 2). All patients recovered to within 90% of their baseline FEV_1 prior to discharge from the site.

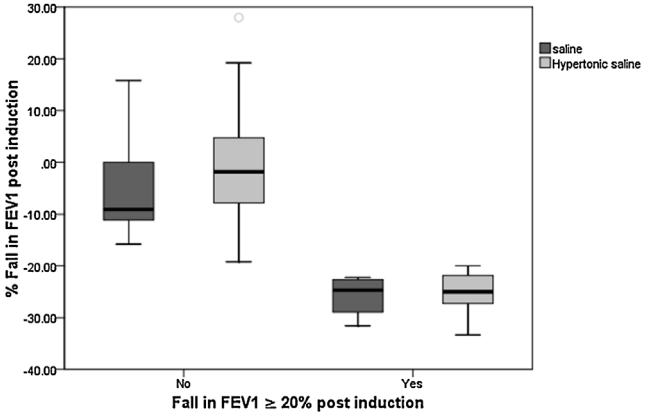

Fig. 1 – Fall in FEV_1 post sputum induction in patients receiving normal saline and hypertonic saline, $p = 0.116$.

The safety of sputum induction was assessed in varying GOLD severity of COPD. There was more than 20% fall in FEV_1 in 6.3% of mild COPD patients compared to 8.5% and 18.9% in moderate and severe COPD, respectively (see Table 3); yet all gave adequate sputum following reversal. We also assessed the correlation of reversibility of FEV_1 on the day of sputum induction to percentage fall in FEV_1. A statistically significant inverse correlation existed, $r = -0.437$, $p = 0.001$, see Fig. 2A, but there was no correlation between percentage fall in FEV_1 and postbronchodilator FEV_1 (ml). There was a good correlation between postbronchodilator FEV_1 and postsaline FEV_1, $r = 0.958$, $p = 0.001$; see Fig. 2B. A stepwise multivariable linear regression model with age, sex, and reversibility as independent predictors of fall in FEV_1 post sputum induction showed only reversibility to be the independent predictor with a coefficient of determination, $R^2 = 0.137$, $p = 0.001$.

Table 2 – Sputum induction parameters.

	Whole group	Males	Females	*p* value
SI pre-FEV_1 ml	1057.6 ± 407.2	1149.5 ± 450.8	915.4 ± 278.0	0.003*
SI post BD FEV_1 ml	1169.8 ± 430.9	1286.9 ± 473.9	996.5 ± 283.6	0.001*
SI postsaline FEV_1 ml	1123.1 ± 444.7	1235.7 ± 488.6	956.7 ± 306.0	0.001*
Overall fall in FEV_1	66 (57.8)	41 (60.3)	25 (54.3)	0.71

Data are presented as mean ± SD; data in parentheses are presented as *n* (%). FEV_1 = forced expiratory volume in one second, FVC = forced vital capacity, BD = bronchodilator, SI = sputum induction.
* Statistically significant.

Table 3 – Sputum induction parameters in GOLD severity of COPD.

n	Mild	Moderate	Severe	Very severe	*p* value
	16	59	37	2	
SI pre-FEV_1 ml	1485 ± 481.3	1125 ± 331.5	775.7 ± 259.2	740 ± 254.5	0.001*
SI post BDFEV_1 ml	1655 ± 472.1	1244.1 ± 354.3	860.3 ± 257.3	820 ± 311.1	0.001*
SI postsaline FEV_1 ml	1557.5 ± 495.4	1209.3 ± 376.0	818.5 ± 297.5	740 ± 311.1	0.001*
Overall fall in FEV_1	11 (68.8)	32 (54.2)	21 (56.8)	2 (100)	0.463
Grade of FEV_1 fall					
>20%	1 (6.3)	5 (8.5)	7 (18.9)	0 (0)	0.35
10–20%	6 (37.5)	8 (13.6)	9 (24.3)	1 (50.0)	0.182
<10%	4 (25.0)	19 (32.2)	5 (13.5)	1 (50.0)	
No drop	5 (31.3)	27 (45.8)	16 (43.2)	0 (0)	

Data are presented as mean ± SD; data in parentheses are presented as *n* (%). FEV_1 = forced expiratory volume in one second, BD = bronchodilator.
* Statistically significant.

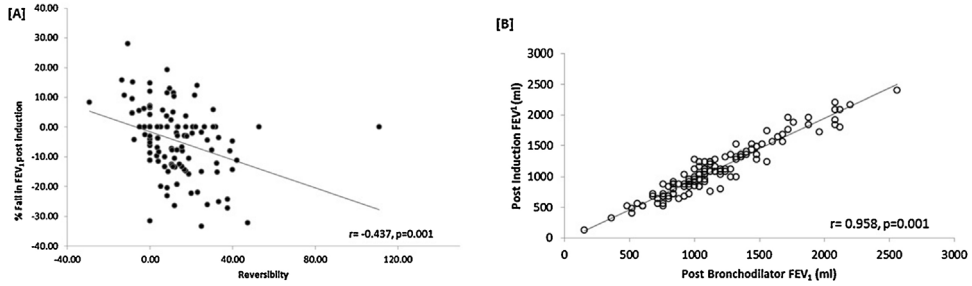

Fig. 2 – (A and B) Relationship between reversibility (ml) and percentage fall in FEV_1 post sputum induction and post bronchodilator FEV_1 and post induction FEV_1.

4. Discussion

This is probably the first study reporting the safety of sputum induction in COPD patients in an Indian population where induced sputum studies are not routinely employed in clinical practice. The study shows that sputum induction is a successful and a safe procedure when performed as per standard guidelines by trained staff. We had a success of 98.28%, while D'Silva et al. reported that sputum induction is successful in almost all patients of COPD and 80% of asthma patients.[8]

We observed that 13 (11.4%) patients with COPD had excessive bronchoconstriction (fall in $FEV_1 \geq 20\%$), while 42.1% had no fall in FEV_1. The overall fall in FEV_1 was 11.1% (5.1, 15.2) with a maximum fall of 33%. A study by Pizzichini et al. reported a maximum fall of 29.7%,[17] and Vlachos-Mayer also reported a maximum fall of 29.5%.[18] All of our patients tolerated the procedure, and no patient complained of breathlessness, wheezes, or chest discomfort. Similar to our study, Rytila et al. also demonstrated a >20% fall in 11% of the COPD patients with no major side effects.[19] All patients with a fall ≥20% recovered to at least 90% of the prebronchodilator level following inhalation of 200 mcg of salbutamol.

71.9% of COPD patients had reported wheeze prior to sputum induction; however, pretreatment with salbutamol helped them tolerate the procedure. We used a relatively low output ultrasonic nebulizer where significant bronchoconstriction rarely occurred.[20]

We studied the fall in FEV_1 in varying grades of GOLD COPD severity and found no statistically significant difference. We observed significantly low values in pre- and postbronchodilator FEV_1 (ml) in female patients, but the percentage fall in FEV_1 was similar in both sexes. There were 18 (15.7%) COPD patients who had $FEV_1 < 800$ ml and they received normal saline nebulizations for sputum induction. The fall in FEV_1 was not statistically different from those who received hypertonic

saline. This supports the fact that normal saline does help in giving adequate sputum for analysis without significant safety issues when FEV_1 is low.

The fall in FEV_1 during sputum induction was inversely correlated with reversibility in FEV_1 (ml) ($r = -0.437$), which was similar to the findings of Rytila et al.,[19] while Wilson AM et al. reported a weak correlation of −0.37.[21] We also determined which factors might predict excessive fall in FEV_1. In multivariate analysis including age, gender, and reversibility, we found only reversibility on the day of sputum induction to be a significant independent predictor of fall in FEV_1 accounting for approximately 13.7% of the variance. This explains that patients of COPD with better response to bronchodilator will have lesser fall in FEV_1.

This procedure is reported to be successful even in hospitalized patients with acute exacerbations of COPD[22–24] and also safe in adolescents with severe asthma.[25]

5. Conclusion

We conclude that sputum induction is successful and safe in varying severity of COPD without causing significant or prolonged fall in FEV_1. Even a drop in $FEV_1 > 20\%$ is reversible with inhaled salbutamol. However, the procedure should be performed following the safety standards by trained staff. This understanding of the safety of sputum induction in COPD patients will help influence clinical practice guidelines for better diagnosis and management of COPD and the asthma COPD overlap syndrome.

Conflicts of interest

The authors have none to declare.

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Original Article

Serum levels of soluble urokinase plasminogen activator receptor (suPAR) as a marker of tuberculosis treatment efficacy

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ABSTRACT

Background: Upon diagnosis, Pulmonary Tuberculosis patients are treated for TB for a period of 6–9 months. At present, there exists very little indication of the efficacy of the particular treatment. A few previous studies have shown that soluble urokinase plasminogen activator receptor (SuPAR) may be used as treatment efficacy marker. SuPAR is a cellular receptor for serine protease urokinase plasminogen activator (uPA). Bacterial endotoxins and cytokines of the innate immune system stimulate the secretion of uPA in monocytes & neutrophils. Serum SuPAR levels are elevated when TB is active and decrease when the patient responds positively to therapy.
Objective: To investigate if SuPAR levels decline upon treatment and whether serum SuPAR levels may be used as a biomarker to monitor Tuberculosis treatment efficacy.
Design: The study was conducted in the department of Biochemistry at VIMS, Ballari, Karnataka. The study subjects were randomly selected from RNTCP centre of VIMS.
Controls: Twelve tuberculin skin test positive healthy controls from the community.
Cases: A total of 60 cases were enrolled for the study and were divided into 3 groups with 20 in each, based on the duration of TB treatment. Group I ($n = 20$): Newly diagnosed pulmonary TB patients before initiation of DOTS. Group II ($n = 20$): TB patients, 2–3 months after initiation of DOTS. Group III ($n = 20$): TB patients who had completed 6 months of DOTS.
Methodology: Hb%, TC, DC(P)%, DC(L)% & ESR were measured by standard procedures. Serum suPAR was measured by the quantitative sandwich enzyme immunoassay technique using the R & D systems Human uPAR Quantikine ELISA Kit.
Results: The suPAR levels were elevated before treatment (3.27 + 2.08 ng/ml) and dropped significantly in groups after 2 months of initiation of therapy (2.18 + 1.17 ng/ml) and after completion of 6 months of treatment (1.50 + 0.93 ng/ml).
Conclusion: The decrease in suPAR levels in PTB patients with treatment is a manifestation of treatment efficacy. Hence suPAR levels can be used to guide clinical decisions in TB management.

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1. Introduction

Tuberculosis (TB) remains one of the world's deadliest communicable diseases.[1] In 2014, 6 million new cases of TB were reported to WHO. Of the estimated 9 million people who developed TB in 2013, more than half (56%) were in the South-East Asia and Western Pacific Regions. India and China alone accounted for 24% and 11% of total cases, respectively.[2,3]

On diagnosis of TB, patients are treated with antitubercular drugs for a period of 6–9 months. The success in controlling TB depends to a great extent on the appropriate diagnosis, the treatment regimen, monitoring, and evaluation. Delayed diagnosis facility, disease transmission, and increase in healthcare costs and mortality cause greater lung damage resulting in chronic disability. As per the recommendation of WHO, currently microscopy and culture are the traditional methods used to monitor the efficacy of TB treatment. But these methods have the limitations of low sensitivity and are time consuming.[4] This urges for a stringent monitoring program that could show the progress of the therapy. In the last decade, several biological markers have been studied. One of the most intensively investigated markers is soluble urokinase plasminogen activator receptor (suPAR).

suPAR is a cellular receptor for serine protease urokinase plasminogen activator (uPA). uPAR can be cleaved from the cell surface by proteases to yield a soluble form of the receptor (suPAR) which has intrinsic chemotactic properties. suPAR plays a role in both innate and adaptive immunity through the fibrinolysis pathway. Bacterial endotoxins and cytokines of the innate immune system stimulate the secretion of uPA in monocytes and neutrophils.[5–7] A few previous studies have shown that suPAR may be used as a TB treatment efficacy marker. Concentrations of the blood plasma protein suPAR are elevated when TB is active and decrease when the patients respond positively to therapy. Hence, the aim of the present study is to investigate whether changes in the serum levels of suPAR can be used to monitor TB treatment efficacy.

2. Material and methods

The study was conducted at the Department of Biochemistry at Vijayanagar Institute of Medical Sciences, Ballari, Karnataka. The study was approved by the Institutional ethical committee. The study subjects were randomly selected from RNTCP centre, VIMS and District Tuberculosis Centre, Ballari. An informed consent was obtained from all the study subjects.

2.1. Controls

Randomly selected twelve tuberculin skin test positive (induration of <3 mm) healthy controls from the community.

2.2. Cases

A total of 60 TB cases were enrolled for the study and were divided into 3 groups with 20 in each based on the duration of starting TB treatment. *Group I* ($n = 20$): Newly diagnosed pulmonary TB patients before initiation of DOTS (Directly observed short course anti tuberculosis therapy) as per WHO guidelines. *Group II* ($n = 20$): TB patients, 2–3 months after initiation of DOTS. *Group III* ($n = 20$): TB patients who had completed 6 months of DOTS.

2.3. Inclusion criteria

(1) Pulmonary TB patients, both males and females in the age group of 20–65 years. (2) Patients with treatment compliance.

2.4. Exclusion criteria

TB patients with HIV/AIDS, pneumonia, diabetes mellitus, cardiovascular disease, malaria, and patients with neoplasms.

2.5. Diagnosis

Both smear-positive and smear-negative PTB patients constituted our study subjects. The smear-negative patients were diagnosed with PTB if clinical signs and X-ray findings were compatible with TB. A detailed history and other laboratory data were obtained from the patients' clinical records. Their age, sex, height, weight, BMI, sputum smear data, and chest X-ray findings were noted. Chest X-rays were taken before therapy began and included standard postero-anterior and lateral views, the results of which were reviewed by a pulmonologist. Lesion width was measured according to chest X-ray grading of disease: (i) minimal lesion, i.e., lesion width less than area restricted to median line, apex, and front costae, solitary lesion located anywhere, and no cavity found; (ii) moderate advanced i.e., width of cavity is less than one lobe, and if a cavity is present, should be in no more than one lobe; and (iii) far advanced corresponding to a lesion width greater than minimal and moderate lesions and, if with cavity, should not exceed a width of 4 cm.[8]

Table 1 – Baseline characteristics of the study subjects.

Study subjects	Age (years)	Sex M:F	Weight (kg)	Height (cm)	BMI (kg/m^2)	Sputum smear Positive: negative	Chest X-ray (width lesion) Minimal: moderate: far advanced
Controls ($n = 12$)	31.91 ± 7.48	9:3	57.5 ± 7.31	155.6 ± 16.6	24.5 ± 4.77	–	–
Group I ($n = 20$)	45.2 ± 18.64	19:1	44.4 ± 8.32	155.2 ± 14.71	22.15 ± 4.25	18:2	7:12:1
Group II ($n = 20$)	37.75 ± 10.90	12:8	47.75 ± 9.72	158.1 ± 12.34	21.65 ± 6.18	17:3	6:12:2
Group III ($n = 20$)	36.95 ± 12.18	17:3	53.15 ± 13.42	137.95 ± 46.45	23.0 ± 4.84	19:1	10:10:0

Table 2 – Comparison of hematological and serum suPAR levels in controls and group I subjects.

Study subjects	Controls ($n = 12$)	Group I (before treatment) ($n = 20$)	p value
Hb%	12.66 ± 1.82	9.44 ± 1.25	<0.0001
TC (cells/cmm)	5066 ± 755	9380 ± 2800	<0.0001
DC (neutrophils)%	67 ± 3.21	61.53 ± 16.56	0.2697
DC (lymphocytes)%	25.91 ± 3.72	30.46 ± 10.10	0.1459
ESR (mm/h)	13.75 ± 1.28	57.7 ± 14.54	<0.0001
Serum suPAR (ng/ml)	1.521 ± 0.466	3.270 ± 2.08	0.0078

Table 3 – Comparison of hematological and serum suPAR levels in Group I and Group II subjects.

Study subjects	Group I (before treatment) ($n = 20$)	Group II (2–3 months after treatment) ($n = 20$)	p value
Hb%	9.44 ± 1.25	10.61 ± 1.45	0.0095
TC (cells/cmm)	9380 ± 2800	7210 ± 2355	0.0116
DC (neutrophils)%	61.53 ± 16.56	68.2 ± 6.91	0.1047
DC (lymohocytes)%	30.46 ± 10.10	28.25 ± 5.07	0.3873
ESR (mm/h)	57.7 ± 14.54	51.5 ± 13.93	0.1766
Serum suPAR (ng/ml)	3.270 ± 2.08	2.18 ± 1.174	0.0483

2.6. *Antitubercular treatment*

Patients were treated according to standard guidelines of the National Tuberculosis program (NTP) consisting of fixed weight-dependent combination of INH (320–400 mg/day), ripampicin (480–600 mg/day), ethambutol (800–1200 mg/day), and pyrazinamide (1000–1250 mg/day) for the two-month intensive phase treatment, followed by rifamficin and INH for the four-month continuation phase.

2.7. *Sample collection*

Under aseptic precautions 4 ml of blood was drawn from each of the study subjects, out of which 2 ml was collected in EDTA bulb for estimation of hemoglobin, total count (TC), differential count (DC) – (neutrophils and lymphocytes), and erythrocyte sedimentation rate (ESR) by standard procedures. Another 2 ml was collected in a plain bulb and allowed to clot for 30 min at room temperature. The sample was centrifuged for 15 min at 1000 × g and the serum aliquots were stored at −20 °C until further analysis for suPAR.

2.8. *Procedure*

Serum suPAR was measured by the quantitative sandwich enzyme immunoassay technique using the R&D systems Human uPAR Quantikine ELISA Kit (www.RnDsystems.com). At present, this kit is for research use only and not for diagnostic purpose for want of FDA approval. The assay was carried out as per the manufacturer's protocol (Catalog Number DUP00). ELISA Plate reading was conducted using a Robonick microplate reader set at 450 nm, with the wavelength correction set at 630 nm.

2.9. *Statistical analysis*

Statistical analysis was done by Students 't' test and one-way ANOVA, which was performed online (www.graphpad.com/quickcalcs) using Graphpad software. A p value of ≤0.05 was considered as statistically significant.

3. Results

3.1. *Patients characteristics*

Sixty cases of pulmonary TB were divided into 3 groups based on duration of anti-TB treatment. Baseline characteristics of the study subjects are shown in Table 1. The study subjects were in the age group of 20–65 years. The number of female Pulmonary TB patients ($n = 15$) was less than the male PTB patients ($n = 45$). BMI was low in PTB patients when compared to controls (22.15 ± 4.25 vs 24.5 ± 4.77).

Table 4 – Comparison of hematological and serum suPAR levels in Group II and Group III subjects.

Study subjects	Group II (2–3 months after treatment) ($n = 20$)	Group III (completion of 6 months of treatment) ($n = 20$)	p value
Hb%	10.61 ± 1.45	11.68 ± 1.87	0.0502
TC (cells/cmm)	7210 ± 2355	7005 ± 2062	0.7712
DC (neutrophils)%	68.2 ± 6.91	67.35 ± 5.68	0.6733
DC (lymphocytes)%	28.25 ± 5.07	31.05 ± 4.91	0.0840
ESR (mm/h)	51.5 ± 13.93	20.5 ± 8.26	<0.0001
Serum suPAR (ng/ml)	2.18 ± 1.174	1.504 ± 0.936	0.0514

Table 5 – Comparison of hematological and serum suPAR levels in Group I and Group III subjects.

Study subjects	Group I (before treatment) ($n = 20$)	Group III (completion of 6 months of treatment) ($n = 20$)	p value
Hb%	9.44 ± 1.25	11.68 ± 1.87	0.0001
TC (cells/cmm)	9380 ± 2800	7005 ± 2062	0.0041
DC (neutrophils)%	61.53 ± 16.56	67.35 ± 5.68	0.1453
DC (lymphocytes)%	30.46 ± 10.10	31.05 ± 4.91	0.8155
ESR (mm/h)	57.7 ± 14.54	20.5 ± 8.26	<0.0001
Serum suPAR (ng/ml)	3.270 ± 2.08	1.504 ± 0.936	0.0013

3.2. *Hematological findings*

The hematological findings and suPAR levels are shown in Table 2. The Hb% was decreased in newly diagnosed PTB patients, but was normal in patients after completion of 6 months of DOTS. There was no significant difference in total WBC count and differential count among the three groups. A significantly high level of ESR was found in Group I (PTB patients before treatment) and also in Group II (2 months after initiation of treatment) when compared to controls ($p < 0.0001$). There was no significant difference in ESR values between Group I and Group II ($p = 0.1766$). But the ESR levels were significantly low in Group III (PTB patients who had completed 6 months of DOTS) when compared to Group II and Group I ($p < 0.0001$), as depicted in Tables 3–5.

3.3. *suPAR levels*

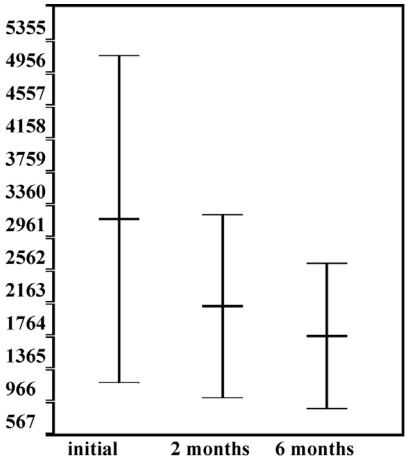
The increase in serum suPAR levels was very statistically significant in Group I patients (median = 2.87 ng/ml, range = 1.09–10.51 ng/ml) when compared to controls (median = 1.34 ng/ml, range = 0.95–2.59 ng/ml) with a p value of 0.0078. The decrease in serum suPAR levels in Group II patients (median = 1.73 ng/ml, range = 0.82–4.72 ng/ml) was statistically significant when compared to Group I patients with a p value of 0.0483. There was further slight decrease in suPAR levels in Group III patients (median = 1.43 ng/ml, range = 0.21–2.52 ng/ml) when compared to Group II (p value = 0.0514). The Group III serum suPAR levels were almost similar to the healthy controls. When one-way ANOVA was applied for analysis of serum suPAR levels in the three groups, the F value was 7.2308 with a probability of 0.0016. Because the probability is less than 0.05, it indicates that there is a statistically significant difference in the mean suPAR values in the three groups as represented in Graph 1.

4. Discussion

TB continues to affect the lives of millions of people worldwide. Lack of accurate method in monitoring TB treatment efficacy could be one of the causes of treatment failure resulting in high morbidity and mortality. The present study aimed to investigate the possible use of suPAR as a TB treatment efficacy marker.

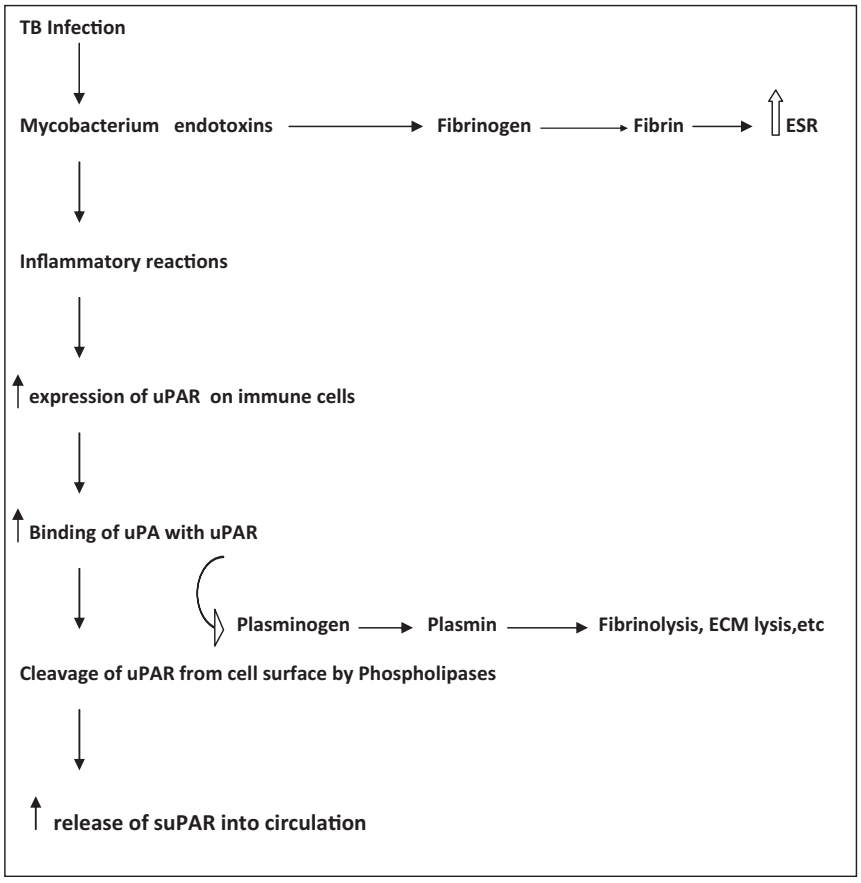
uPAR, also known as CD87 (283 amino acids), is a cell surface receptor that binds urokinase-type plasminogen activator (uPA) with high affinity, thereby facilitating the pericellular activation of plasminogen. uPAR is anchored to extracellular surfaces through a glycosyl phosphatidylinositol (GPI) linkage, with no transmembrane domain. Soluble uPAR is generated by removal of the GPI anchor by an endogenous phospholipase D. suPAR is known to be a chemotactic agent for promoting the immune system. suPAR concentration positively correlates to the activation level of the inflammatory and immune system (Flowchart 1). It is present in plasma, urine, blood, serum, and cerebrospinal fluid. It is a marker of disease severity and aggressiveness.[9] Along with low BMI and high ESR Group I patients (before treatment) showed high serum suPAR levels (range 1.09–10.51 ng/ml). Our findings were in accordance to the study done by Triwahju et al.[10] But the range of suPAR levels in patients with active PTB in a study done in Indonesia[6] was 8.53–28 ng/ml and in African people, it ranged between 0.9 ng/ml and 45 ng/ml.[11] Whether the suPAR levels in different population are genetically influenced needs further investigation.

The suPAR levels were high before treatment (3.27 ± 2.08 ng/ml) and lowered significantly in Group II and Group III, i.e., after 2 months of initiation of therapy (2.18 ± 1.17 ng/ml) and after completion of 6 months of treatment (1.50 ± 0.93 ng/ml). This was in accordance to the studies of Triwahju et al.[10] and Eugen-Olsen.[12] Increased suPAR levels before initiation of treatment may be a result of mobilization of macrophages into the bronchi and the increased immune activation and inflammation caused by the active infection.[13]



source	df	SS	MS	F	P-value
treatments	2	31741871.040	15870935.520	7.2308	0.0016
error	57	125109148.702	2194897.346		
total	59	156851019.742			

Graph 1 – suPAR levels in Group I, Group II, and Group III.



Flowchart 1 – The overall mechanism of Tuberculosis induced increase in ESR and suPAR levels.

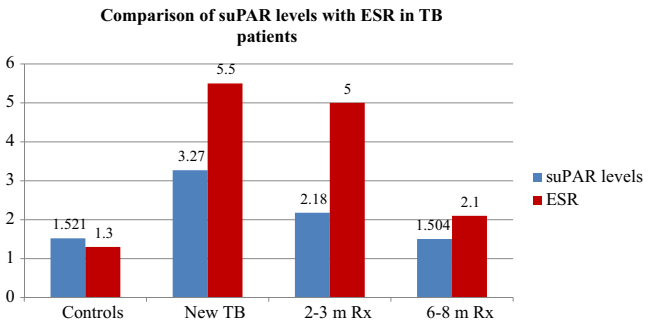
All the 20 patients in Group III, who had completed 6 months of treatment, were symptom-free. The positive effect, i.e., efficacy of anti-tubercular treatment was reflected in decrease in serum suPAR levels.

Also, when suPAR levels were compared with ESR levels in these patients, suPAR levels significantly reduced after 2 months of treatment while there was no significant decrease in ESR levels. ESR levels decreased significantly only after 6 months of treatment (Graph 2). This suggests that suPAR levels are early indicators/markers of TB treatment efficacy, i.e., within 2 months of treatment.

suPAR levels among patients with minimal lesion, moderate advanced lesion, and far advanced lesion did not differ significantly. Similar findings were reported by other authors.[6] But this was in contrast with findings of another study[4] in which suPAR levels correlated significantly with width of the lesion. Triyudani[6] is of the opinion that this could be due to the use of different methods in measuring the lesion.

A number of studies have confirmed that high suPAR levels at the time of initiation of TB treatment and elevated suPAR levels even after 1–2 months of treatment were associated with increased risk of mortality.[4,11,12] A few other studies have shown that suPAR levels were significantly higher in the multi-drug resistance TB patients, possibly due to the survival of a sub-population of bacteria.[8] The value of suPAR for the diagnosis of TB in sputum-negative patients is currently being tested by Virogates (Denmark) in a clinical trial in Guinea-Bissau (study Number: LSSP-CT-2205–012173).[14]

Hence, suPAR levels can be used as early marker to guide clinical decisions in TB management. Decreased suPAR levels after 2 months of treatment when compared to baseline levels suggest that the patient is responding to the treatment. But, when there is persistently raised SuPAR levels, even after 2 months of treatment, this suggests a resistance to primary line of treatment. The physician can then switch the patient to secondary line of treatment immediately without waiting for 6 months to complete the treatment regimen. suPAR is measurable in blood, serum, plasma, and urine using the



Graph 2 – Comparison between suPAR and ESR levels between all the groups.

well-established and inexpensive ELISA method. This method not only allows for a high level of technical simplicity (requiring only simple reagents and an ELISA plate reader), but also allows the test to be widely transportable to the remotest settings.

5. Conclusion

The lowering of suPAR levels in PTB patients after treatment is an indicator of treatment efficacy. suPAR levels were elevated almost 3 times in TB patients before treatment and drastically lowered after the first 2 months of treatment, followed by further decrease at the end of 6 months of treatment. The suPAR levels can predict the prognosis and treatment efficacy by the end of 2 months of intensive phase treatment itself.

Limitations and further scope of the study

The present study was conducted in a small sample of 20 cases in each group. Further follow-up studies can be conducted in large groups to validate our findings.

Authors' contributions

Indumati V: (1) the concept and design of the study, acquisition of data, analysis and interpretation of data, (2) drafting the article and revising it critically for important intellectual content, (3) final approval of the version to be submitted.

Vijay V: (1) Analysis and interpretation of data. (2) Revising the article critically for important intellectual content, and (3) final approval of the version to be submitted.

Krishnaswamy D, Rajeshwari V, and Ramesh A: Revising the article critically for important intellectual content and final approval of the version to be submitted.

Shantala D and Shilpa A: Acquisition of data, analysis, and final approval of the version to be submitted.

Conflicts of interest

The authors have none to declare.

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Original Article

Band pattern analysis of mutations in rifampicin resistance strain of *Mycobacterium tuberculosis* by Line Probe assay in patients from Delhi, India

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Sequencing

ABSTRACT

Background: The GenoType MTBDR*plus*, a commercial Line Probe Assay (LPA) kit from Hain Lifescience, Germany, is endorsed by India's RNTCP Program for diagnosis of DRTB cases among smear-positive sputum samples. Although the LPA has been studied in several laboratories, there is a wide variation in existing *M. tuberculosis* strains across the globe, and false results can occur due to the presence of unique genetic mutations in different settings.
Aim and objective: An attempt was made to carry out band pattern analysis using LPA and also to observe uncommon mutations in MDR strains.
Materials and methods: Sputum samples were collected from MDR suspects and transported to intermediate reference laboratory (IRL) at New Delhi Tuberculosis Centre in Delhi. Sputum decontamination, DNA extraction, amplification, hybridization, and band pattern analysis of Line Probe assay strips was performed as per manufacturer's instructions.
Results: Among the 3000 samples with interpretable LPA strips, rifampicin drug resistance with or without isoniazid was observed in 600 samples. The most common mutation detected by LPA in the rpoB gene was Ser516Leu (29.0%). Novel mutations reported in this study include mutation from CAG (Gin) to CAT (His) at codon 517, AGC (Ser)-AGG (Arg) at codon 512, ACA (Thr) to GCA (Ala) at codon 526, TTG (Leu)-CTG (Leu)[s] at codon 524.
Conclusion: High frequencies of uncommon mutations in rpoB gene by LPA were observed, highlighting possibility of those in-silico detected mutations that may not impart phenotypic resistance further.

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1. Introduction

Globally, tuberculosis is a leading cause of death with increasing rates of drug resistance is of serious concern. Early diagnosis and rapid treatment are the key elements to combat TB and reduce transmission by rendering infectious cases non-infectious.

Multi-drug resistant tuberculosis (MDR-TB) defined as the resistance to at least rifampicin (RIF) and isoniazid (INH) poses grave challenge because of prolonged, limited and expensive treatment options with 10–30% of cases resulting in failure of treatment and death.

Worldwide, 9.6 million people are estimated to have fallen ill with TB in 2014. Globally, 12% of the 9.6 million new TB cases in 2014 were HIV-positive. To reduce this burden, detection and treatment gaps must be addressed, funding gaps closed and new tools developed. In 2014, 6 million new cases of TB were reported to WHO, fewer than two-thirds (63%) of the 9.6 million people estimated to have fallen sick with the disease. This means that worldwide, 37% of new cases went undiagnosed or were not reported. Of the 480,000 cases of multidrug-resistant TB (MDR-TB) estimated to have occurred in 2014, only about a quarter of these – 123,000 – were detected and reported.[1]

Since, conventional drug susceptibility testing (DST) requires a lot of resources such as infrastructure and trained manpower. WHO has recommended the use of molecular Line Probe assays (LPAs) for rapid detection of MDR[2] because conventional phenotypic DST takes 4–6 weeks from the receipt of samples in laboratory. Commercially available genotype MTBDR*plus* assay detects mutations associated with both rifampicin and isoniazid resistance simultaneously.

Unlike RIF resistance, in which 95% of isolates have mutations within an 81-bp region of the *rpoB* gene encoding the RNA polymerase β subunit,[3] isoniazid (INH) resistance has been associated with mutations in several genes.[4,5]

Moreover, since this technique is polymerase chain reaction (PCR) based, it allows detection of low levels of resistant bacteria amidst a predominantly susceptible population, providing a more accurate representation of the susceptibility of the infecting bacteria.[6,7] Different mutations lead to varying degrees of resistance and influence bacterial ability to multiply.[8]

As per recent studies report, the use of sophisticated techniques such as DNA sequencing to detect drug resistance mutations which can serve as epidemiological markers, since the relative frequency of alleles associated with resistance varies geographically.[9–11]

Present study was, therefore, undertaken to evaluate the performance of MTBDR*plus* for the detection of MDR in pulmonary tuberculosis (PTB) patients in Delhi and also to fulfill the objectives which encompass determination of the nature and frequency of mutations associated with resistance to both INH and RIF.

2. Materials and methods

This study was carried out from specimens received from January 2015 to December 2015, at New Delhi Tuberculosis Centre, IRL-STDC, in New Delhi. This Intermediate reference laboratory is certified by Health Ministry to perform these tests for the Proficiency Testing programme. This lab is participating in regular Proficiency Testing programme conducted by National Reference Laboratory (NRL). Clearance for the study was obtained from the Institutional Ethics committee and Institutional Scientific Committee.

Patients with sputum smear positive results under the RNTCP approved diagnostic criteria that is, Criteria A, B, and C. Patients within the 14–70 years age group will be considered.

Two sputum samples (spot and early morning) were collected in 50 ml wide-mouth sterile falcon tube in the field and transported at IRL on the same day from suspects registered with the DOTS (Directly Observed Therapy Short Course) centers of the RNTCP (Revised National Tuberculosis Control Programme) in 14 associated chest clinics. A unique laboratory accession number was provided to each sample.

Staining and microscopy: Smears were prepared from two sputum samples and were directly stained by Auramine O Fluorescent staining, and were microscopically examined.[13] From each suspect, the specimen with the highest smear grading based on RNTCP guidelines was tested by LPA.

Decontamination: The sputum specimens were processed in class II type A2 bio-safety cabinet in a bio-safety level III (BSL-3) laboratory and were decontaminated by N-acetyl-L-cysteine and sodium hydroxide (NALC-NaOH) method.[14] Subsequently, the sediments were suspended in 2 ml sterile phosphate buffer (pH 6.8) for further testing. Two 500 μl aliquot of the processed sputum deposits were made, where in one was used for the LPA test, and the other was used for MGIT liquid culture.

3. Initial MDR screening with Line Probe assay

Line Probe assay was conducted as per the manufacturer's instruction. The procedure followed is given below briefly:

a) **DNA extraction:** DNA extraction was performed with the Genolyse version 1.0 kit from the processed specimen.
b) **PCR amplification:** PCR was performed on ABI Thermocycler using the following protocol – first, the template DNA was denatured for 15 min at 95 °C, followed by 10 cycles consisting of 30 s at 95 °C and 2 min at 58 °C, with an additional 30 cycles consisting of 25 s at 95 °C, 40 s at 53 °C and 40 s at 70 °C. The final cycle consisted of an 8 min run at 70 °C.
c) **Hybridization and detection:** Hybridization was performed using the hybridization Kits, including reagents and 12 well plastic tray and instrument (Twincubator) as provided by the manufacturer.

Interpretation of results: As per the manufacturer's product insert, in order to give a valid result, all six expected control bands should appear correctly otherwise, the result is considered invalid. In general for the three loci, a pattern comprising only WT (wild type) bands was interpreted as sensitive. Resistance was interpreted as: (i) absence of one or more WT bands, (ii) presence of mutant bands with or (iii) without the simultaneous absence of the complementary WT.[15]

Table 1 – Drug concentrations for MGIT DST procedure.

Drug	Concentration of the drug after reconstitution	Volume added to the MGIT tube	Final concentration of the MGIT tube
STR	83 μl/ml	100 ml	1.0 μl/ml
INH	8.3 μl/ml	100 ml	0.1 μl/ml
RIF	83 μl/ml	100 ml	1.0 μl/ml
ETB	415 μl/ml	100 ml	5.0 μl/ml

Keys: STR – streptomycin, INH – isoniazid, RIF – rifampicin, ETB – ethambutol, μl – microlitre, ml – milliliter.

The simultaneous presence of WT and corresponding mutant bands was referred to as a mixed pattern. After interpretation of the LPA results, MDR was defined as resistance to at least INH and RIF. Monoresistance – as resistance to only one drug and polyresistance – as resistance to two or more drugs excluding the INH-RIF combination simultaneously.

Culture: Decontaminated specimen (0.5 ml) was inoculated on to MGIT 7H9 broth medium for MGIT 960 liquid culture.[14] MGIT tubes were automatically examined in the MGIT system. Smears were made from positive liquid culture tubes and stained by ZN staining for confirmation of acid-fast bacilli (AFB). Immuno-chromatographic Lateral flow assay (SD Bio-line) was done on all positive MGIT tubes with positive AFB results prior to DST for confirmation of *M. tuberculosis* complex. MGIT tubes with inoculated samples were kept for incubation up to six weeks and if no growth was detected, and declared as culture negative.

Drug susceptibility testing by liquid culture method: All 1–5 days old liquid MGIT positive *M. tuberculosis* culture was used for DST. 0.5 ml of this suspension was used for testing of four first line drugs (SM, INH, RIF, EMB) and 1:100 dilution for growth control was used. BACTEC 960 SIRE Supplement (0.8 ml) was added aseptically to each of the labeled MGIT DST tubes. Aseptically reconstituted STR drug (0.1 ml) in the STR labeled tube and similarly other drugs were added in the respective drug tubes (Table 1). For batch Quality control, a known pan-sensitive H37Rv strain of *M. tuberculosis* was used.

DNA sequencing: Twenty five DNA isolates were randomly selected for sequencing which were showing both types of results like discordant as well as some concordant results (between conventional phenotypic MGIT DST and genotypic LPA DST). DNA sequencing was performed, by dideoxy-terminator cycle sequencing kit and later analyzed by ABI Prism automated sequencing instrument. 1 ml of positive culture from MGIT 7 ml tubes which were positive for *M. tuberculosis* complex and also showing no growth on Brain Heart Infusion agar media (sterile) was added to 250 μl of 1× TE buffer and then heat killed at 80 °C for 45 min.

Genomic DNA extraction: Genomic DNA was extracted by CTAB (cetyl-trimethyl ammonium bromide) method, using 10% CTAB-NaCl.[16]

Electrophoresis: Gel electrophoresis was done on 1% agarose gel to confirm the presence of quality of DNA. The samples were electrophoresed at constant 100 V for 40 min (Fig. 1).

Primers used: Amplification of rpoB gene including the 81 bp hot spot region was carried out by using customized primers. These primers were designed using the SG Primer program.[17] Primers containing 18–20 bp of *M. tuberculosis* sequence were designed to amplify the region of interest. One primer in each set also contained a 40- to 50-bp GC-rich portion (GC clamp) at its 5′ end to serve as the highest melting domain.[17] The GC clamps, with a melting temperature of 95 °C, prevent the strands of a PCR fragment from separating completely (Fig. 2).

The sequence data were analyzed by using the sequencing analysis software, version-5.2. The nucleotide sequence obtained was analyzed using Basic Local Alignment Search Tool (BLASTn). The sequence was further subjected for BLASTx to know the amino acid change in comparison with wild type

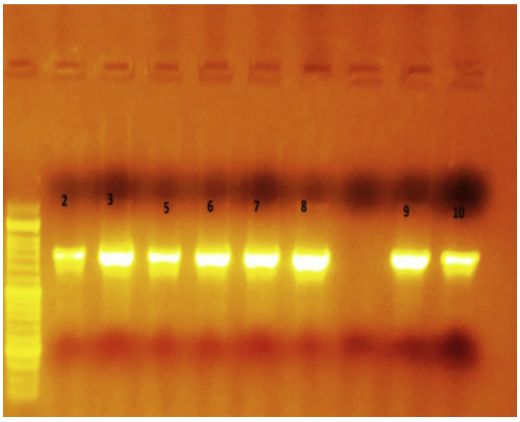

Fig. 1 – 1% agarose gel image showing amplified DNA in culture isolates. The quantity (concentration) and quality (optical density value) of extracted DNA was checked for the A60/A280 ratio on a spectrophotometer.

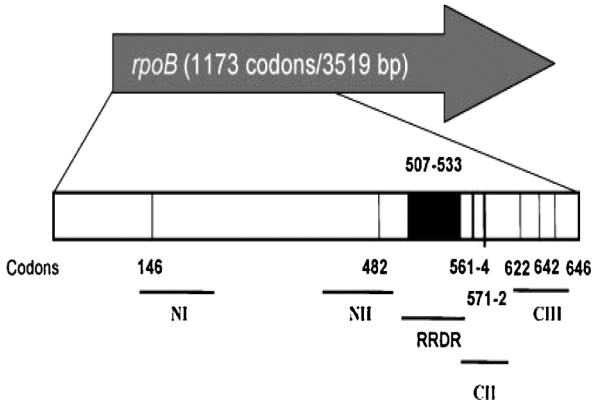

Fig. 2 – Regions of rpoB scanned for RIF resistance mutations. The NI, NII, RRDR, CII, and CIII fragments that were amplified to scan for mutations in rpoB are shown. Codon numbers refer to alterations that were previously reported for RIF-resistant isolates.[3,4,18]

Table 2 – LPA results among different grading of sputum smear.

LPA results	Smear grading (FM) (N = 3000)			
	3+ (N = 617) 20.6%	2 + (N = 993) 33.1%	1+ (N = 910) 30.3%	Sc+ (N = 480) 16%
Sensitive = 2013 (67.1%)	415	666	610	322
MDR = 482 (16.1%)	99	160	146	77
MTBC negative = 12 (0.4%)	2	4	4	2
Mono-R RIF = 118 (3.9%)	24	39	36	19
Mono-R INH = 375 (12.5%)	77	124	114	60

Keys: FM – fluorescent microscopy, RIF – rifampicin, INH – isoniazid.

M. tuberculosis (H37Rv). The nucleotide changes were confirmed for the amino acid codes referred from the following figure showing amino acid table.

Repeat testing: Sputum specimens resulting in inconsistent development of bands on the MTBDR*plus* strip and/or if no *M. tuberculosis* control band appeared, underwent repeat PCR and hybridization from the extracted DNA. Isolates with discordant results of the LPA and the MGIT C&DST were sent for sequencing.

Data analysis: Patterns of RIF and INH resistance in MDRTB and non MDRTB isolates were analyzed. The ability of RIF resistance alone to predict MDR was also investigated. Results obtained through phenotypic DST and the Genotype MTBDR*plus* were compared for RIF, INH and MDR for concordance and/or discordance if any. For calculation, the reference DST result for RIF was the MGIT DST result. An analysis of banding patterns associated with RIF and INH resistance in MDR-TB and non MDR-TB strains was performed.

The sensitivity, specificity, predictive value positive (PPV), predictive value negative (NPV), and overall accuracy of LPA results were compared to the conventional MGIT DST results for RIF and INH, and the ability of RIF resistance alone to predict MDR. These were calculated using free online statistical calculators available at http://www.medcalc.org/calc/.

4. Results

Genotypic DST: Among the 3000 samples with interpretable LPA results, rifampicin drug resistance was observed in 600 cases with the highest smear grading sample only. 482 (16.1%) were MDR-TB, 118 (3.9%) had Rif mono-resistance. 375 (12.5%) had Inh mono-resistance, and 2013 (67.1%) were sensitive to both drugs. 12 (0.4%) were negative for *M. tuberculosis*. There was a significantly higher likelihood of obtaining an interpretable MTBDR*plus* result from a specimen with a positive smear grade compared to those specimens with a scanty smear grading (Table 2).

Drug resistance associated mutations: As per the band pattern obtained in LPA, the most common mutation detected in the rpoB gene was S531L (61.4%), diagnosed by the presence of MUT3 band. There was no significant difference in the prevalence of this mutation in MDR-TB specimen and R mono-resistance specimen. 17 (3.4%) cases had heteroresistance mutations, 13 had S531L and 4 had D516V mutations. Of the overall 600 samples, in whom R-resistance was detected, 169 were on the basis of missing Wild type probes and did not have any positive mutant probe (Table 3).

5. Mutations identified using the Genotype MTBDR*plus* assay

The frequency of mutations detected in the rpoB gene is described in Table 3.

Phenotypic DST: All of the 600 rifampicin resistant samples were put up in MGIT liquid culture. Culture positive were 531 (88.5%), 32 (5.4%) were contaminated, 37 (6.1%) were culture negative. Phenotypic DST by MGIT 960 was done for 510 out of

Table 3 – The frequency of mutations detected in the rpoB gene.

S#	Band pattern presence (+) or absence (–)				# of cases (% observed)
	Wild type band	Gene region	Corresponding mutation band	Mutation	
1	WT8(–)	530–533	MUT3(+)	S531L	296 (61.4%)
2	WT7(–)	526–529	MUT2A(–), MUT2B(–)	H526Y, H526D	46 (9.5%)
3	WT3(–), WT4(–)	513–517, 516–519	MUT 1(–)	D516V	44 (9.1%)
4	WT8(–)	530–533	MUT3(–)	S531L	31 (6.4%)*
5	WT2(–)	510–513	MUT(–)	L511P$^{\Delta}$	17 (3.5%)*
6	WT2(–), WT3(–)	510–513, 513–517	MUT(–), MUT1(–)	D516V	14 (2.9%)*
7	WT8(+)	530–533	MUT3(+)	S531L	13 (2.6%)^{HT}
8	WT7(–), WT8(–)	526–529, 530–533	MUT2A(–), MUT2B(–)	H526Y, H526D	9 (1.8%)
9	WT1(–)	506–509	MUT(–)	–	4 (0.8%)*
10	WT8(+)	530–533	MUT1(+)	D516V	4 (0.8%)^{HT}
11	WT5(–), WT6(–)	518–522, 521–525	MUT(–)	–	3 (0.6%)*
12	WT4(–), WT5(–)	516–519, 518–522	MUT1(–)	D516V	1 (0.2%)*

Keys: WT – wild type, MUT – mutation, ^{HT} – heteroresistance, * – uncommon mutations, $^{\Delta}$ – this rare mutation has only been detected theoretically (in-silico) as per the MTBDR*plus* kit insert.

Table 4 – Correlation of R and H resistance on LPA DST with LC DST.

S#	LPA DST	MGIT 960 DST				LPA DST
		Rifampicin resistant	Rifampicin sensitive	Isoniazid resistant	Isoniazid sensitive	
1	Rifampicin resistant	246	22	258	3	Isoniazid resistant
2	Rifampicin sensitive	28	204	80	119	Isoniazid sensitive
3	Total	274	226	338	122	Total
4	Concordance: 95%	Lower limit	Upper limit	Concordance: 78%	Lower limit	Upper limit
5	Sensitivity: 90%	87%	92%	Sensitivity: 76%	75%	77%
6	Specificity: 90%	87%	93%	Specificity: 97%	93%	99%
7	Positive predictive value: 92%	89%	94%	Positive predictive value: 99%	97%	100%
8	Negative predictive value: 88%	85%	91%	Negative predictive value: 60%	57%	61%

531 samples which were positive for *M. tuberculosis* complex and showing rifampicin resistance in LPA only. Amongst these 510 isolates, 480 (94%) were MDR-TB, 3 (0.6%) had Rif mono-resistance. 5 (1%) had Inh mono-resistance and 20 (4%) were sensitive to both drugs and 2 (0.4%) were negative for *M. tuberculosis*. Overall, a total of 483 patients were detected with RIF resistance by MGIT C&DST, whereas 600 were detected by LPA i.e. an additional 117 (19.5%) R-resistant cases were identified by LPA.

Concordance between MGIT LC & DST and LPA DST: A total of 483 patients had both LC and LPA DST results available. Initial analysis showed agreement of results in 454 (94%) patients, including 246 with R-resistance and 204 with RIF susceptibility on both LPA and LC media (initial concordance 94%; sensitivity: 93% (CI: 88–96%); specificity: 94% (CI: 88–97%); positive predictive value: 95% (CI: 90–97%); negative predictive value: 92% (CI: 86–96%)) (Table 4).

There were 50 (10%) specimens with discordant rifampicin DST results between LC DST and LPA. These included 28 results that were RIF resistant on LC DST and susceptible on LPA, and 22 that were RIF susceptible on LC DST and resistant on LPA (Table 4). Repeat liquid culture and DST were

Table 5 – Mutations in *M. tuberculosis* rpoB gene detected by DNA sequencing.

S#	Affected region	Codons[a]	ORFcodon(s)[b]	Amino acid change[c]	Base change(s)[d]	Confirmed
1	N1 region	146–410	176	Gin to Leu	CAG Δ CTT	1^{HT}
2			177	Leu to Leu	CTG Δ CTG	$7^{S,HT}$
3			189	Tyr to Tyr	TAC Δ TAC	$2^{S,HT}$
4			195	Arg to Arg	CGA Δ CGA	$1^{S,HT}$
5			212	Lys to Lys	AAG Δ AAG	$4^{S,HT}$
6	RRDR region	507–533	511	Arg to Arg	AGG Δ AGG	$1^{S,HT}$
7			511	Leu to Leu	CTG Δ CTG	7^{S}
8			511	Leu to Pro	CTG Δ CCG	1
9			512	Ser to Arg	AGC Δ AGG	$\mathbf{1}^{N}$
10			513	Ser to Ser	AGC Δ AGC	1^{S}
11			515	Met to Ile	ATG Δ ATT	1
12			516	Asp to Tyr	GAC Δ TAC	4
13			516	Asp to Val	GAC Δ GTC	3
14			517	Gin to His	CAG Δ CAT	$\mathbf{1}^{N}$
15			522	Ser to Ser	TCG Δ TCT	1^{S}
16			524	Leu to Leu	TTG Δ CTG	1^{NS}
17			526	Thr to Ala	ACA Δ GCA	$\mathbf{1}^{N}$
18			526	His to Gin	CAC Δ CAA	1
19			526	His to Asp	CAC Δ GAC	2
20			531	Ser to Trp	TCG Δ TGG	1
21			531	Ser to Leu	TCG Δ TTG	3
22	C2 region	534–561	535	Pro to Ser	CCG Δ TCG	1
23			537	Gly to Gly	GGT Δ GGA	1^{S}
24			538	Leu to Met	CTG Δ ATG	1
25			542	Val to Leu	GTG Δ TTG	1
26			553	Ser to Leu	TCG Δ TTG	1
27			556	Gly to Gly	GGC Δ GGT	1^{S}

[a] Codons are given relative to *E. coli* rpoB (accession no. AAC43085) using a BLASTP alignment (Altschul et al., 1997) against Rv0667.
[b] *M. tuberculosis* rpoB (Rv0667) codon numbering.
[c] The first amino acid changed to the second amino acid indicated as Δ, ^{HT} indicates heteroresistant and ^{S} indicates silent mutations. Underline for double mutations.
[d] Nucleotides of altered codons are underlined.

performed for these discordant samples, as per the repeat testing procedure.

In relation to INH, the analysis showed agreement of results in 377 (78%) patients, including 258 (68.5%) with INH-resistance and 119 (31.5) with INH-susceptibility on both LPA and LC & DST (sensitivity: 72% (CI: 65–78%); specificity: 97% (CI: 89–99%); positive predictive value: 99% (CI: 95–99%); negative predictive value: 54% (CI: 45–63%)). There were 83 (22%) specimens with discordant INH DST results between LC&DST and LPA (Table 4).

DNA sequencing: Each of these 25 specimens selected randomly was further subjected to sequencing of rpoB gene. Eighteen distinct DNA alterations within the RRDR were detected in these isolates, and a total of 13 distinct mutations were detected in the N1 and C2 region in rpoB gene from forward set only (Table 5). These mutations include a 3-bp insertion, small deletions (2–3 nucleotides), mostly single nucleotide substitutions, alterations of two adjacent nucleotides, and double mutations in separate codons.

Novel mutations reported in this study include mutation from CAG (Gin) to CAT (His) at codon 517, AGC (Ser)-AGG (Arg) at codon 512, ACA (Thr) to GCA (Ala) at codon 526, TTG (Leu)-CTG (Leu)[s] at codon 524. Since the Line Probe assay detects both alterations and hence both changes may contribute to the drug resistance phenotype.

6. Patients with uncommon mutation results are being followed for clinical correlation with treatment outcome

Discussion: To the best of our knowledge this study thoroughly focuses on band pattern analysis to investigate genotypic profiles in the *rpoB*, *katG* and *inhA* regions associated with drug resistance using the MTBDR*plus* assay. Techniques which detect MDR mutations in new cases at onset or during therapy would enable rapid identification of MDR and facilitate the modification of regimens with improvement to programme practices. The value of RIF as a surrogate MDR marker has been documented[19] and further corroborated in our study also.

Additionally resistance can be inferred from the absence of a wild type signal alone, without confirmation by a mutant probe signal (in 28.2% of our isolates) and may be due to a mutation in a region not associated with resistance.[21] Such susceptible isolates would be called resistant leading to the unnecessary removal of RIF and/or INH from therapy. This highlights the need for the interpretation of genotypic data in conjunction with patient clinical status and the determination of mutations specific to certain geographical locales.

As reported widely elsewhere, phenotypic Rifampicin resistance was strongly associated with mutation in the 81 base pair region of rpoB targeted in the LPA assay.[22,23] In this study, the most commonly observed mutations were in the region of rpoB 530–533, mostly S531L mutation. This is similar to the findings of in a South African study.[24] In this patient population of consecutively-enrolled smear positive TB patients suspected of having MDR-TB, the prevalence of rifampicin resistance was unsurprisingly high.

The routine use of LPA can substantially reduce the time to diagnosis of R and/or H-resistant TB, and can hence potentially enable earlier commencement of appropriate drug therapy and thereby facilitate prevention of further transmission of drug resistant strains. This confers a major advantage to this test. Unacceptable delays in obtaining both culture and DST results by these conventional methods were commonplace, specifically in drug resistant cases. A number of patients may be ''lost'' due to default and/or death whilst awaiting the availability of the DST results. The study also highlights that the availability of rapid diagnostics at central laboratories needs to be supplemented with rapid specimen transportation mechanisms.

Overall, the concordance between the methods for INH and RIF ranged from 86–97% and 94–99% respectively. Of 22 isolates that were MTBDR*plus* RIF resistant but MGIT DST sensitive, 18 lacked the WT8 band, including 16 which showed the MUT3 (S531L). Though associated with high level resistance, its detection could imply a smaller proportion of resistant bacteria. Low level resistance which may remain undetected despite conventional DST has been previously reported.[21,25]

The selection of the method used to detect mutations associated with drug resistance can be challenging. Since DNA sequencing of the amplified product not only detects but also identifies the specific mutation, this method serves as the gold standard. However, the frequency and nature of mutations in the rpoB gene among RMP-resistant *M. tuberculosis* strains vary considerably according to the geographical location or the ethnic populations.[11,26,27]

Our study has a limitation to find any association between a particular mutation and the occurrence of monoresistance or MDR. However, other studies have reported a significantly higher level of *katG* and S531L mutations in MDR isolates compared to INH or RIF monoresistant isolates respectively.[7,20] It is likely that this difference is due to the relatively low occurrence of S531L and the proportions of rpoB, *katG* and *inhA* mutations in our study setting. Secondly, DNA sequencing was done for 25 samples only due to shortage of facilities.

Conclusion: Overall, high frequencies of uncommon mutations in rpoB gene by LPA were observed, highlighting possibility of those in-silico detected mutations that may not impart phenotypic resistance further. Thorough analysis of band pattern in LPA strips need to be documented and correlated with phenotypic results if necessary. Our results also provide evidence for the need of DNA sequencing for confirmation of true resistant cases.

Authors' contributions

All authors read and approved the final manuscript for publication. HV designed, performed and drafted the manuscript under the guidance of MH. KKC, AK, DS have reviewed the study and manuscript.

Conflicts of interest

The authors have none to declare.

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Radiology forum

Diagnosis at a glance of radiological imaging

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ABSTRACT

A 14-year-old boy was referred to our outpatient department with complaints of dry cough, high-grade fever for 20 days and chest X-ray (CXR) opacity. The clinical examination was normal. His CXR showed a right mid-zone lobulated, homogenous opacity. Computed tomography (CT) of thorax showed a well-defined cystic lesion in right upper lobe with 5–10 HU (Hounsfield unit) suggesting a fluid filled cyst. CT guided aspiration of cyst was undertaken and revealed clear fluid with no cells. Post-aspiration high-resolution CT images demonstrated classical signs of hydatid cyst.

A 14-year-old boy hailing from Nepal was referred to our outpatient department with complaints of dry cough, high grade fever for 20 days and chest X-ray (CXR) opacity. The clinical examination was normal. His CXR showed a right mid-zone lobulated, homogenous opacity. Computed tomography (CT) of thorax showed a well-defined cystic lesion in right upper lobe with 5–10 HU suggesting a fluid filled cyst (Fig. 1). CT guided aspiration of cyst was undertaken and revealed clear fluid with no cells. Post-aspiration high-resolution CT images demonstrated classical signs of hydatid cyst. These signs were "the dancing hand sign" described as membranes dancing on the remaining cyst fluid (Fig. 2), "the serpent sign" described on internal rupture of cyst with parasitic membranes falling to the bottom of cyst and assuming a serpentine shape (Fig. 3), "the mass within cavity sign" appearance due to crumpled membranes falling to the most dependent portion of the cavity after complete evacuation of cyst fluid (Fig. 4) and "the empty cyst sign" seen after complete evacuation of parasitic membranes from the cyst (Fig. 5), chest radiograph PA view (Posterio-anterior view) of the patient (Fig. 6). The patient was started on oral albendazole (400 mg) twice daily and on follow-up is symptomatically better.

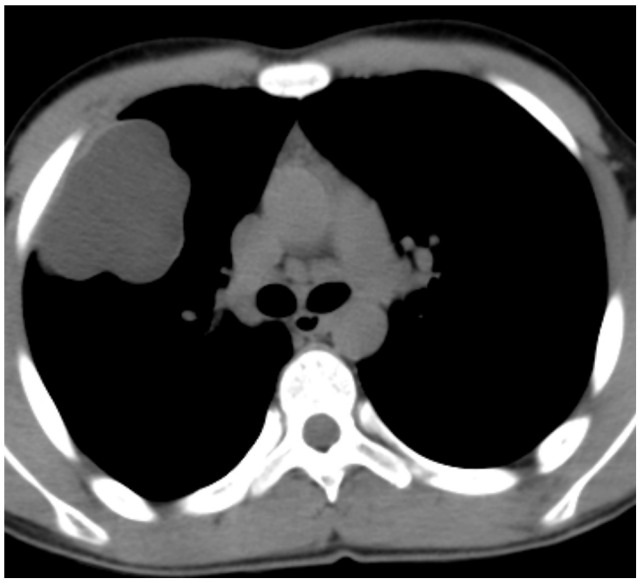

Fig. 1 – Pre-aspiration CT thorax showing a well defined cystic lesion in right upper lobe with 5–10 HU suggesting a fluid filled cyst.

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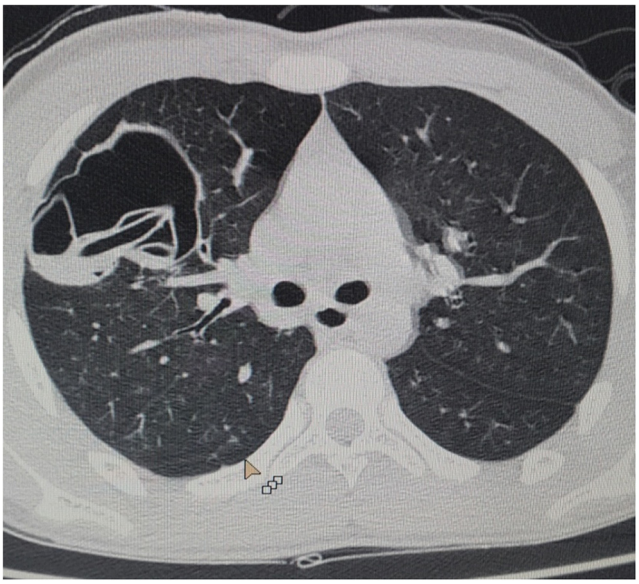

Fig. 2 – Post-aspiration CT thorax showing the dancing hand sign.

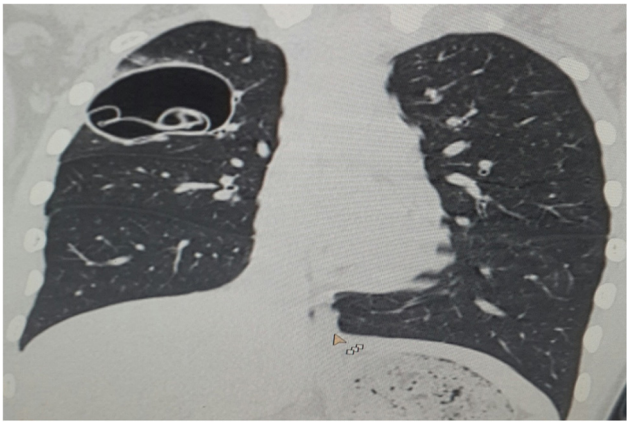

Fig. 3 – Post-aspiration CT thorax showing the serpent sign.

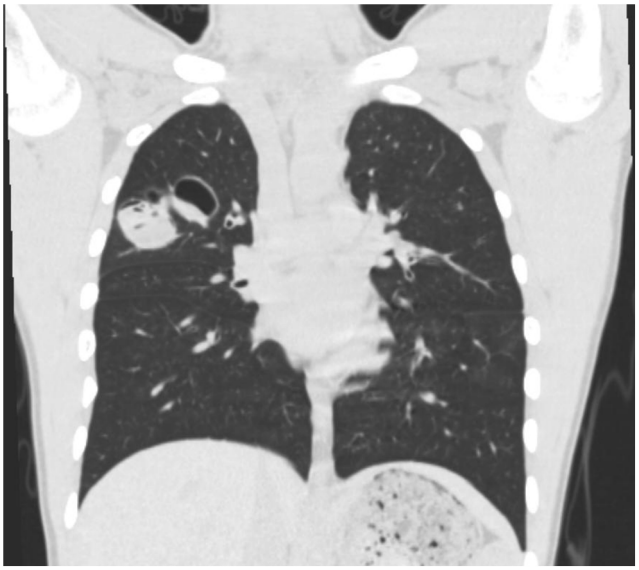

Fig. 4 – Post-aspiration CT thorax showing the mass within cavity sign.

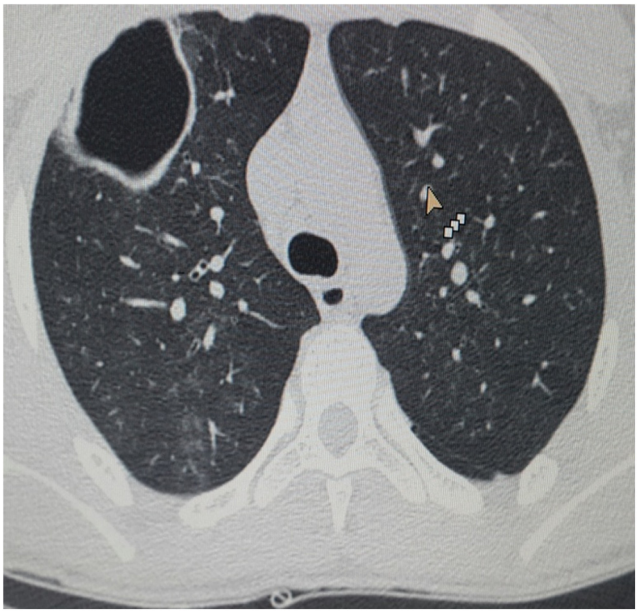

Fig. 5 – Post-aspiration CT thorax showing the empty cyst sign.

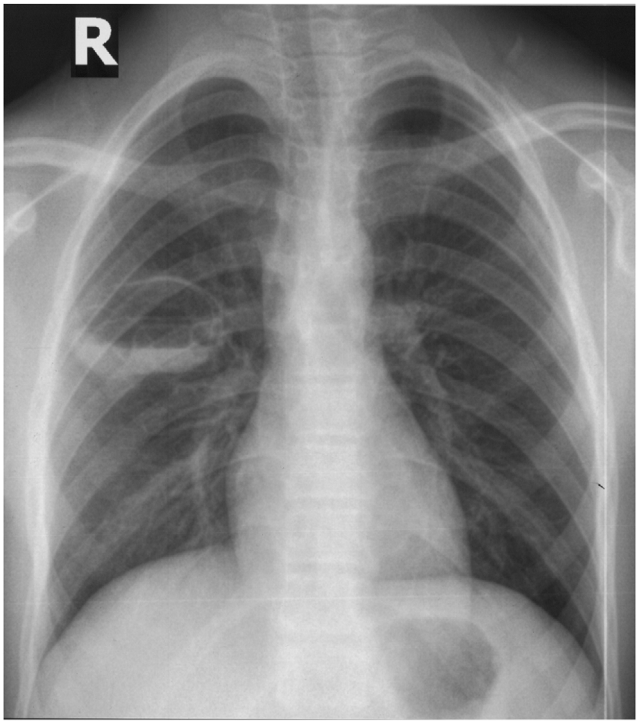

Fig. 6 – Postero-anterior view of the chest radiograph.

Conflicts of interest

The authors have none to declare.

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Case Report

Sternoclavicular joint tuberculosis: A series of 9 cases

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ABSTRACT

Tuberculosis (TB) is a chronic disease that may affect any part of the human body. Though the osteoarticular TB is not uncommonly seen, TB of the sternoclavicular joint (SC joint) is an infrequently reported condition. The very fact that many physicians have never seen a single case of SC joint TB in their entire career makes them never think of this condition in cases of chronic swellings of the medial end of clavicle. We are reporting here our experience with nine cases of SC joint TB that were treated by us. Delay in diagnosis in each of the case was a common feature, and they had been treated in line of inflammation elsewhere. Diagnosis was arrived at by clinical, radiological, and microscopic examinations. Six of the reported cases responded well to antitubercular chemotherapy, and in one of the cases, chemotherapy was combined with debridement, which was actually done during biopsy and primarily for tissue diagnosis; in another two cases, immunomodulation therapy for HIV was given along with antitubercular therapy. Tuberculous etiology should be considered for patients presenting with atypical sites of skeletal inflammation, and a high index of suspicion by the treating physician is necessary to make early diagnosis and appropriate treatment.

1. Introduction

Tuberculosis (TB) is still a very common disease in developing countries and is being increasingly reported in developed countries in immunodeficient population. In addition, the increasing prevalence of TB in both immunocompetent and immunocompromised individuals makes TB a topic of universal concern.[1,2] Skeletal TB constitutes around 10% of the extrapulmonary cases, with weight-bearing joints being most commonly involved.[1] TB of the spine is a commonly reported condition along with that of the hip and knee joints. Sternoclavicular joint (SC joint) involvement has been reported in <1% of osteoarticular TB cases.[1–4] The condition usually starts from the medial end of the clavicle as a painful swelling of insidious onset and gradual progression.[1,5] These cases may be seen in patients with active TB or even in patients having foci of TB involving other joints. Despite the availability of advanced diagnostic facilities, TB of the SC joint often raises diagnostic problems either because of uncommon site of involvement or a lack of awareness of this condition among the treating physicians, and because of this, these are frequently misdiagnosed or diagnosed at a late stage.[4]

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We are here reporting a series of nine cases of unilateral TB of SC joint with or without any demonstrable active foci of pulmonary or extrapulmonary TB.

2. Patients and treatment

Between 2005 and 2013, we treated 9 patients with tubercular arthritis of SC joint (Table 1). Their mean age was 41 (24–65) years. Involvement of right side was there in six patients while one patient also had associated multicentric spinal involvement, which was previously reported by us.[7] The most common presentation was pain and swelling on the medial end of clavicle in six patients. One patient had painless swelling at the medial end of clavicle while discharging sinus was the presentation in two patients. Mild restriction of motion of the shoulder because of pain was found in 2 patients and neck pain was reported by one of the patients. Constitutional symptoms in the form of malaise, fever, and loss of weight and of appetite were seen in 6 patients. The mean duration of symptoms before diagnosis was 6 (2–13) months. 6 patients were initially treated by physicians for chest, neck, or shoulder pain, and were referred to us after the onset of swelling or discharging sinus. Hematological evaluation in all patients showed a raised erythrocyte sedimentation rate and a positive Mantoux test.

Plain radiographs of the chest and the medial end of the clavicle were unremarkable in all cases, except in one patient where a doubtful cystic lesion was noted at the medial end of the clavicle; in another patient, evidence of active disease in lungs along with multicentric spinal involvement was noted. Magnetic resonance imaging (MRI) or computed tomography (CT) scan was done in 7 patients. The final diagnosis was arrived at in these cases on the basis of clinical examination, suspicious radiographs, and representative tissue biopsy. Fine-needle aspiration biopsy was performed in every patient and open biopsy was performed in 1 patient where fine-needle aspiration cytology (FNAC) was non-conclusive. Evidence of caseous granulomatous pathology was obtained in five cases by FNAC and by open biopsy in one case. Acid-fast bacilli (after Ziehl–Neelsen staining) were seen in only 3 cases. TB polymerase chain reaction (PCR) of aspirated fluid was positive in 4 of our cases. One patient had an active tubercular lesion in the lungs and two of the cases tested positive for HIV.

At the time of open biopsy, which was done in one patient, debridement of the joint was done and all dead tissue removed. After confirmation of diagnosis, all the patients were put on an antitubercular regimen (antitubercular therapy—ATT) consisting of four drugs—rifampicin (10 mg/kg daily), isoniazid (5 mg/kg daily), ethambutol (15 mg/kg daily) and pyrazinamide (25 mg/kg in divided doses)—in the initial intensive phase for 3–6 months. After that, depending on the clinical response to treatment, patients were switched over to three drugs (isoniazid, rifampicin and ethambutol), followed 1 or 2 months later by the omission of ethambutol. Isoniazid and rifampicin were continued as maintenance therapy for 14–18 months. Final outcome of therapy was judged by clinical, hematological, and radiological parameters. Seven of the patients showed evidence of local healing of the lesion within 6 months of appropriate treatment. Two patients were given ATT along with immunomodulation therapy for HIV infection; one did not respond to first-line ATT, and was found to have drug resistance to isoniazid and rifampicin, and was treated with second-line antitubercular drugs (Figs. 1–4).

Table 1 – Summary of 9 cases.

	Age/sex/side	Chief complaints	Associated disease	Diagnosis	Treatment
Case 1	53/male/right	Pain and swelling since 5 months	None	Caseating granulomatous lesion on biopsy, PCR positive	Antitubercular therapy for 16 months
Case 2	24/female/right	Pain and swelling since 2 months	HIV positive	AFB in ZN staining with positive PCR	Antitubercular therapy for 14 months with immunomodulation therapy for HIV
Case 3	51/male/left	Discharging sinus since 4 months	None	AFB in ZN staining	Antitubercular therapy for 18 months
Case 4	32/male/right	Pain and swelling since 7 months	HIV positive	Caseating granulomatous lesion on biopsy with positive PCR	Second-line antitubercular therapy for 18 months with immunomodulation therapy for HIV
Case 5	58/female/left	Painless swelling since 13 months	Rheumatoid arthritis	Caseating granulomatous lesion on biopsy	Antitubercular therapy for 18 months
Case 6	45/male/right	Pain and swelling since 6 months	None	Caseating granulomatous lesion on biopsy	Antitubercular therapy for 16 months
Case 7	65/male/right	Discharging sinus since 6 months	None	Caseating granulomatous lesion on biopsy	Antitubercular therapy for 12 months
Case 8	33/female/left	Pain and swelling since 8 months	None	Caseating granulomatous lesion on biopsy with positive PCR	Antitubercular therapy for 15 months
Case 9	54/male/right	Pain and swelling since 5 months	None	AFB in ZN staining	Antitubercular therapy for 18 months

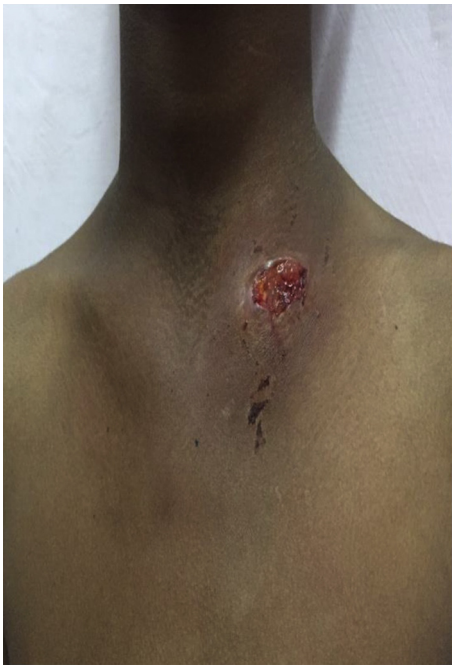

Fig. 1 – Clinical picture showing discharge from medial end of clavicle.

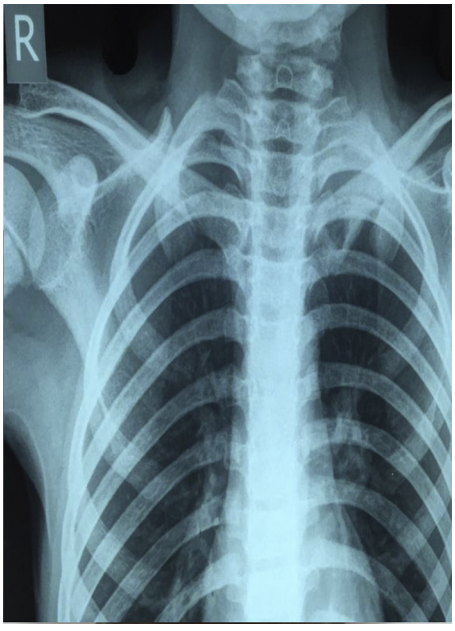

Fig. 2 – Chest radiograph showing lytic lesion in medial end of clavicle.

3. Discussion

With the advent of acquired immunodeficiency syndrome and multidrug resistant strains of Mycobacterium tuberculosis, there is resurgence of TB all over the world and TB has been described to affect almost any part of the body with impunity.[5] The incidence is not expected to stay so low in the near future due to the emergence of multidrug-resistant strains and the rapid increase in the number of immunocompromised patients.[9] Two of our patients were positive for HIV infection, one was diabetic and another one was a rheumatoid-positive female.

However, TB rarely involves the SC joint, leading to frequent delayed diagnosis and misdiagnosis,[1,2,11] as most of our cases were treated with ordinary analgesics or antibiotics because of misdiagnosis; this rarity of occurrence of TB in the SC joint can be attributed to the peculiar blood supply of this joint.[5] Diagnosis is often delayed by several weeks or months due to the absence of constitutional symptoms, unusual site, and indolent nature of the disease.[3,5] Early diagnosis is essential to prevent further complications including compression or erosion of the large blood vessels at the base of the neck and migration of the tuberculous abscess to the mediastinum in case of TB of SC joint.[5]

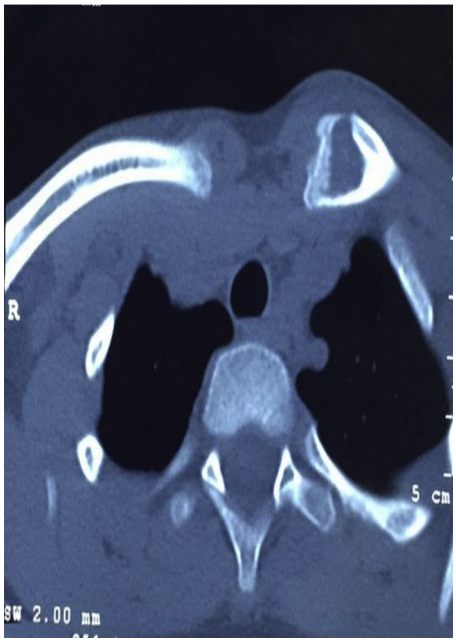

Fig. 3 – CT scan showing expansile lytic lesion with arthritis of SC joint.

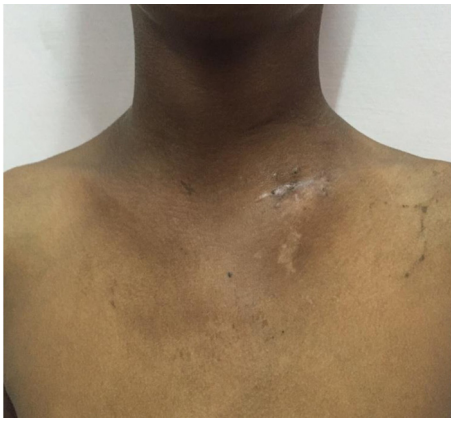

Fig. 4 – Clinical picture with healed lesion.

Clinical presentation of sternoclavicular TB varied considerably in patients reported by us. It has been found that sternoclavicular TB may follow either a more aggressive course leading to a painful, destroyed joint or a slowly progressive, relatively painless disease without constitutional symptoms and minimal joint destruction due to differences in the virulence of the organisms and host resistance. The most common presenting complaints in decreasing order of frequency were pain and swelling, swelling alone, or a discharging sinus. Pain and swelling were the presenting complaints in six of our patients, painless swelling in one, and a discharging sinus in two cases, in which both were immunocompromised. The common differential diagnosis of sternoclavicular TB includes low-grade pyogenic abscess, rheumatoid disease, myeloma, and secondary deposits.[5]

The pathogenesis of SC joint infection is not fully understood. Some investigators believe that chest wall TB occurs by reactivation of latent foci formed during hematogenous or lymphatic dissemination of primary TB, while others opine that it occurs by direct extension from contiguous lung/pleura.[2–6] Only one of our cases had overt evidence of active pulmonary TB.

Conventional plain radiographs are often not helpful in diagnosing this pathology.[2,3] CT and MRI of the site may show osseous destruction of the clavicle, sternum, and SC joint but are not specific for the condition.

Shah et al.[8] suggested that all radiological and imaging modalities are complementary but MRI is the best technique for early detection and diagnosis of SC joint TB. In our case series, MRI confirmed a lytic lesion on the medial end of the clavicle along with sclerosis and a collection of fluid in the SC joint extending into the subcutaneous plane. These MRI findings were highly suggestive of TB. MRI is a radiation-free modality with excellent delineation of soft tissue pathology, and is very helpful to define the extent of the disease and is considered as best imaging modality for early detection and diagnosis of SC joint TB.[9]

The final histological and microbiological confirmation of SC joint TB requires FNAC or an open biopsy. Presence of Acid Fast Bacilli (AFB) in Zeihl-Neelsen (ZN) staining was demonstrated in three of our cases, and the rest of the cases were diagnosed by the presence of epithelioid cell granuloma with multinucleated giant cells and caseous necrosis in the tissue specimen or positive TB PCR. PCR is a recent technique with a specificity ranging from 92% to 98% allowing early diagnosis.[10,11] Surgical debridement at the time of open biopsy promoted early healing, which was done in one of our case; all other cases in our series were treated with ATT alone or along with immunomodulation therapy for HIV infection. Duration of therapy in our series ranged from 14 to 18 months.

In conclusion, possibility of tuberculous etiology should be kept in mind in any patient with chronic destructive arthritis in an unusual location such as SC joint in an endemic region and susceptible population. A high index of suspicion is mandatory for timely diagnosis and treatment and non-responders to ATT must be looked for drug resistance or an immunocompromised state.

Conflicts of interest

The authors have none to declare.

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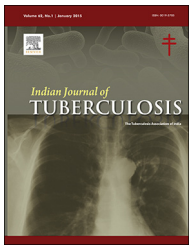

Case Report

Multiple abdominal abscesses – A not so common presentation of NTM

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ABSTRACT

Non-tuberculous *Mycobacteria/Mycobacterium* other than tuberculosis (MOTT) are ubiquitous organisms. They are acid fast bacilli often giving trouble to the physician to distinguish it from *Mycobacterium tuberculosis*. These organisms are a menace for the treating physician as when to treat and when not to treat. They are often difficult to diagnose and may present in a variety of forms with propensity to cause number of infections of different body parts and organs. They are more common in immunocompromised individuals e.g. HIV infection. Here we are reporting a not so common manifestation of NTM which presented as multiple abdominal abscesses in a middle aged female probably secondary to surgical site infection, however she responded dramatically to the designed treatment.

1. Introduction

Non-tuberculous mycobacteria (NTM) are found in the environment in soil, water, milk, food, aerosols, and wild and domestic animals. NTM include *Mycobacterium avium* complex (MAC), *M. kansasii*, rapidly growing *Mycobacteria*, *M. flavescens*, *M. scrofulaceum*, *M. szulgai*, *M. gordonae*, *M. chelonae* and others. NTM has propensity to cause a number of infections, including pulmonary, lymphatic, skin, soft tissue, skeletal and catheter related infections.

NTM infections are relatively common in patients with acquired immune deficiency syndrome (AIDS) and especially in those with a CD4T lymphocyte cell count <50 cells/μl; however, they can also occur in other individuals.

Although NTM can cause serious pulmonary and disseminated infection in some patients, soft tissue infection in the form of abdominal abscesses is not so commonly reported.

Reported here is a patient who presented with multiple abdominal abscesses infected with NTM (*M. chelonae*).

2. Case report

A 40-year-old female presented in the OPD with complaints of multiple abdominal abscesses, low grade fever, poor appetite for the past 8–9 months. She has been visiting various doctors for the same and was given different type of treatment including antibiotics, antifungals and also first line antitubercular drugs but the symptoms and lesions persisted. On

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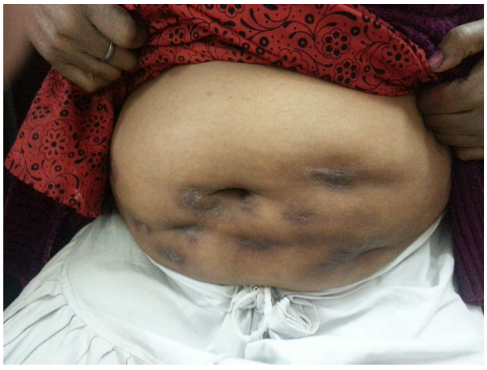

Fig. 1 – Patient's picture on first visit.

further going deep into the history, she told that she was operated for some ovarian cyst/benign lesion around one and a half year back however the records were not available.

On examination patient had multiple abscesses on the anterior abdominal wall with blackish discoloration, some of them were having discharge, pus was aspirated and was sent for smear acid fast bacilli, culture of *Mycobacterium tuberculosis*, culture of *Mycobacterium* other than tuberculosis (MOTT), Gram stain and culture sensitivity for pyogenic organisms. Apart from it patient was also advised Routine investigations including chest X-ray, hemogram, and blood sugar.

Meanwhile, the patient was kept on symptomatic and supportive treatment along with broad spectrum antibiotics. Smear came out to be positive for acid fast bacilli and subsequently culture report demonstrated growth of NTM and the species was *M. chelonae* (the report was obtained for two occasions from different abscesses). Patient was subsequently started on injection amikacin, oral clarithromycin, oral clofazimine and oral levofloxacin. Patient tolerated the regimen well and significant improvement in the abdominal lesions was seen after around three months of the initiation of treatment, her appetite also improved and she became afebrile. Patient tolerated the regimen well with no significant side effects, she was again evaluated at 6 months and there was further healing of the lesions with disappearance of the blackish discoloration and levelling of the abscesses. Injection amikacin was stopped and rest of the regimen was continued, patient is doing well till date with almost complete healing of the lesions and left with few residual scar marks (Figs. 1 and 2).

3. Discussion

M. chelonae, like many NTM, are ubiquitous in the environment and have been isolated from both natural and potable water sources, soil, contaminated solutions and reptiles.[1] It is hydrophobic, adheres to surface, and is resistant to chlorine and some other detergents.[2]

M. chelonae causes disease sporadically, as well as in patients with identifiable risk factors. It most commonly causes infection of skin and skin structures, localized cellulitis, subcutaneous abscess or as disseminated disease.[3] It has caused outbreaks of skin infections associated with tattoo parlours. Disseminated infection has been seen associated with organ transplants, diabetes mellitus, malignancy, corticosteroid use and other immunosuppressants.[4] It has been associated with surgical site infections,[5] peritonitis and dialysis catheter infections.

Eye is the second most common site of infection. Organism is known to cause dacryocystitis, canaliculitis, conjunctivitis, endophthalmitis, scleritis and keratitis.[6] Pulmonary infection is common with other NTM like MAC, *M. kansassii* and *M. abscessus*. *M. chelonae* induced pulmonary infection is uncommon but can occur in patients with underlying lung disease such as cystic fibrosis or bronchiectasis. Sinusitis and otitis media have been reported, musculoskeletal involvement is uncommon.

The first national survey evaluating the presence of NTM in United States was done between 1981 and 1983 and it showed an annual prevalence of 1.78 NTM cases/100,000 persons, with *M. chelonae*/abscessus reported as 0.08/100,000 persons.[7] NTM lung disease was studied in hospitalized patients between 1998 and 2005 in 11 states and showed increasing prevalence with age in men and women, with some variation between states.[8] In Oregon study (2005–2006) NTM prevalence was found to be 7.2/100,000, out of which *M. chelonae* was 0.2/100,000.[9]

Overall it appears that NTM prevalence is increasing in certain populations and geographic areas but the degree of contribution of *M. chelonae* is uncertain, it represents a smaller percentage of these infections than other NTM species.

Most *M. chelonae* infections may resolve before treatment is rendered, but chronic non-resolving infections require antimicrobial therapy guided by appropriate identification and susceptibility testing. Empiric therapy should be avoided except in unusual circumstances. Macrolide antibiotics are the cornerstone of therapy; however, development of resistance

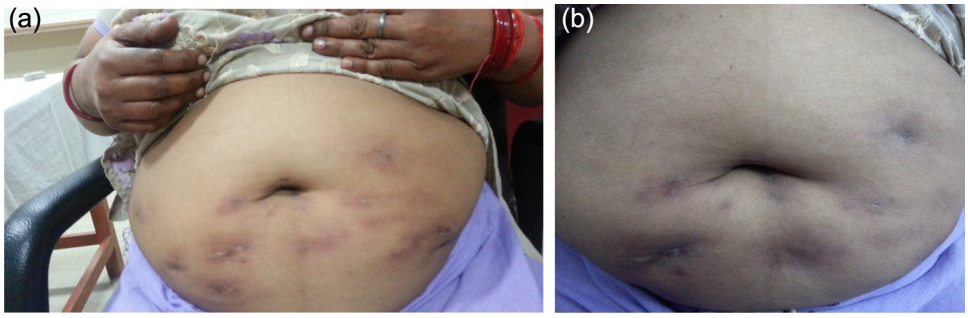

Fig. 2 – Patient's pictures at subsequent visits. (a) Three months after treatment. (b) Twelve months after treatment.

with prolonged monotherapy has been reported but it is less common with aminoglycoside.

Linezolid, fluoroquinolones and clofazimine[10] are few other drugs with good in vitro activity against the organisms.

Treatment duration is at least 6 months in disseminated disease and 12 months of negative sputum cultures in case of lung infection.

Conflicts of interest

The authors have none to declare.

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Case Report

Unusual case of coexistent pulmonary cryptococcosis and tuberculosis in an immuno-competent host

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ABSTRACT

Coexistence of pulmonary cryptococcosis with other infections has commonly been described in immuno-suppressed individuals. In immuno-competent hosts, such coexistence is rare and mostly described in disseminated disease or uncommonly involving different sites. The simultaneous coinfection of cryptococcosis and tuberculosis of lung in an immuno-competent host is extremely rare with only one previously reported case in the literature. This is the second such case and the first to be reported in India. We describe a case of a 36-year-old immuno-competent male who presented with haemoptysis and cough. Computed tomography showed a sub-pleural lung nodule. Diagnostic thoracoscopic wedge resection of the right lung nodule revealed granulomatous inflammation with cryptococcus on histopathology. Coexistent tuberculosis was diagnosed by microbiological culture study on lung tissue. The patient responded clinically to fluconazole and anti-tubercular therapy. This case shows that although rare, coexistent infections can occur in immuno-competent persons and highlights the importance of careful evaluation and tissue microbiological culture examination.

1. Introduction

Pulmonary cryptococcosis is a common opportunistic fungal infection in acquired immunodeficiency syndrome (AIDS) and other immuno-suppressed patients. Coexistence of pulmonary cryptococcosis and tuberculosis in an immuno-competent host is very rare.

2. Case report

A 36-year-old male, resident of Delhi, India, presented to the chest medicine out patient department with chief complaints of cough and haemoptysis for 10 days. The haemoptysis was mild in the form of blood-stained sputum and was associated with dry cough. There was no history of fever, weight loss, breathlessness or associated chest pain and no neurological symptoms were present. There was no past history of hypertension, diabetes mellitus or any other systemic disease. No history of any previous lung disease or history of any medication was present. The patient was a non-smoker with no significant travel history. Prior to this episode, the patient was absolutely healthy. At presentation, the patient was conscious and well oriented. Pulse rate was 80 per minute, blood pressure was 126/84 mm of Hg and respiratory rate was 15 per minute. There was no fever, pallor, cyanosis, oedema or any peripheral lymphadenopathy. Respiratory, central

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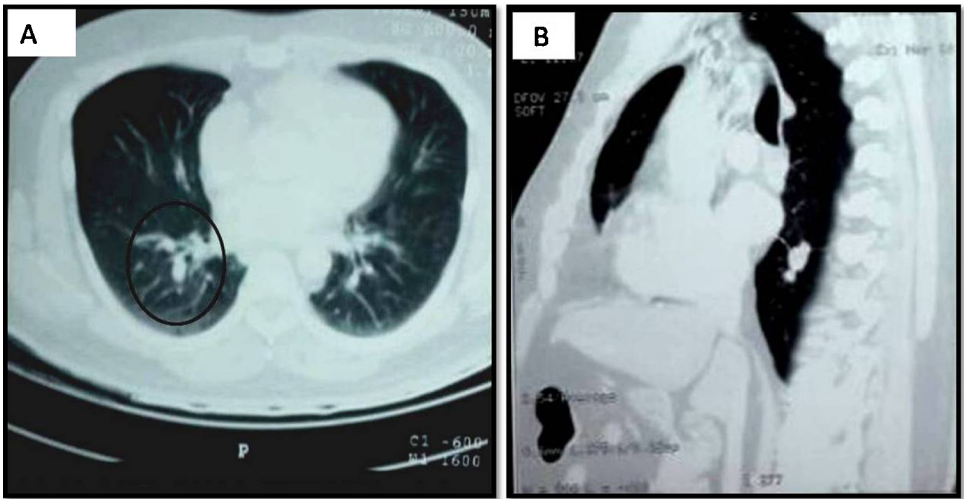

Fig. 1 – Computed tomography (CT) scan of thorax showing a parenchymal nodule in the lung (A – AP view and B – lateral view).

nervous, cardiovascular and abdominal systemic examinations were within normal limits. His haemoglobin was 12.9 g%, the total leucocyte count was 8200/cu mm and differential count was within normal limits. The ESR was mildly increased (45 mm/1st hour) and serum C-reactive proteins were mildly elevated (20 mg/L). Other serum biochemical profiles such as liver function tests, renal function tests and serum electrolytes were within normal limits. Serology for human immunodeficiency virus (HIV) was negative. Mantoux test revealed an induration of 10 mm × 12 mm at 48 h. Echocardiogram was normal. Chest X-ray did not show any significant abnormality. Computed tomography (CT) scan of thorax revealed a bilobed parenchymal nodule with radiating margins measuring 2.5 cm × 1.3 cm in the medial basal segment of right lower lobe of the lung in the sub-pleural location (Fig. 1A and B). Positron emission tomography scan showed increased flurodeoxy-glucose uptake in the lung lesion along with mediastinal and abdominal lymphadenopathy with the largest node measuring 2.7 cm × 1.3 cm. The radiological findings were suggestive of infective aetiology. The possibility of lymphoma however could not be excluded. Three consecutive sputum samples were negative for acid-fast bacilli (AFB). Cytology of bronchoalveolar lavage yielded scant material and no organisms or tumour was identified. A diagnostic thoracoscopic wedge resection of the right lung nodule was done and the tissue was sent for both histopathological and microbiological studies. Grossly, lung tissue showed a small sub-pleural nodule measuring 2.5 cm × 1 cm × 1 cm, which was grey white and necrotic. Microscopic examination showed an abscess cavity with dense acute and chronic inflammatory infiltrate. There were numerous spherical, budding yeast forms of cryptoccocus present both intracellularly within the histiocytes and giant cells as well as extracellularly. These were encapsulated, with narrow-based budding, and were positive with silver stain for fungus. The capsule was highlighted by mucicarmine stain. The surrounding lung showed chronic interstitial inflammation and multiple epithelioid cell granulomas with Langhans type of giant cells and focal necrosis (Fig. 2A, B and D). Lymph nodes also showed granulomatous inflammation (Fig. 2C). Stain for AFB was negative in both lung and lymph nodes. The histopathological diagnosis was consistent with pulmonary cryptococcosis with granulomatous inflammation of lung and lymph nodes. On reviewing the patient's clinical history, he was found to have long-term exposure to pigeon droppings. He was put on antifungal treatment consisting of oral fluconazole 200 mg/day. Culture for fungus on tissue identified *Cryptococcus neoformans*. Subsequently, his rapid AFB culture at the end of 3 weeks turned out to be positive for Mycobacterium tuberculosis. Hence, this case was of coexistent pulmonary cryptococcosis and tuberculosis in an immuno-competent patient. In view of associated tuberculosis diagnosed on microbiological culture studies, anti-tubercular treatment was added, which comprised of isoniazid, rifampicin, pyrazinamide and ethambutol for first two months followed by two drugs, i.e. rifampicin and isoniazid, for another four months. The patient responded to treatment, and at last follow-up at the end of 6 months, he was well with complete resolution of symptoms and no lesion on contrast enhanced computed tomography chest.

3. Discussion

Cryptococcosis is a common opportunistic fungal infection seen usually in AIDS patients. However, its occurrence in immuno-competent patients is relatively uncommon. In most cases, it is known to be associated with AIDS, but has also been found in other types of immuno-compromised, non-HIV states, which include immuno-suppressive drug treatment, malignancies, cirrhosis and diabetes mellitus. Uncommonly, it can occur in the absence of an apparent immune deficiency. Kiertiburanakul et al. in their 17-year review found that cryptococcosis is not rare in HIV-negative patients.[1] This organism has a worldwide distribution and is often found in soil contaminated by pigeon excreta. Prevalence of this infection has shown an increase over the last two decades.

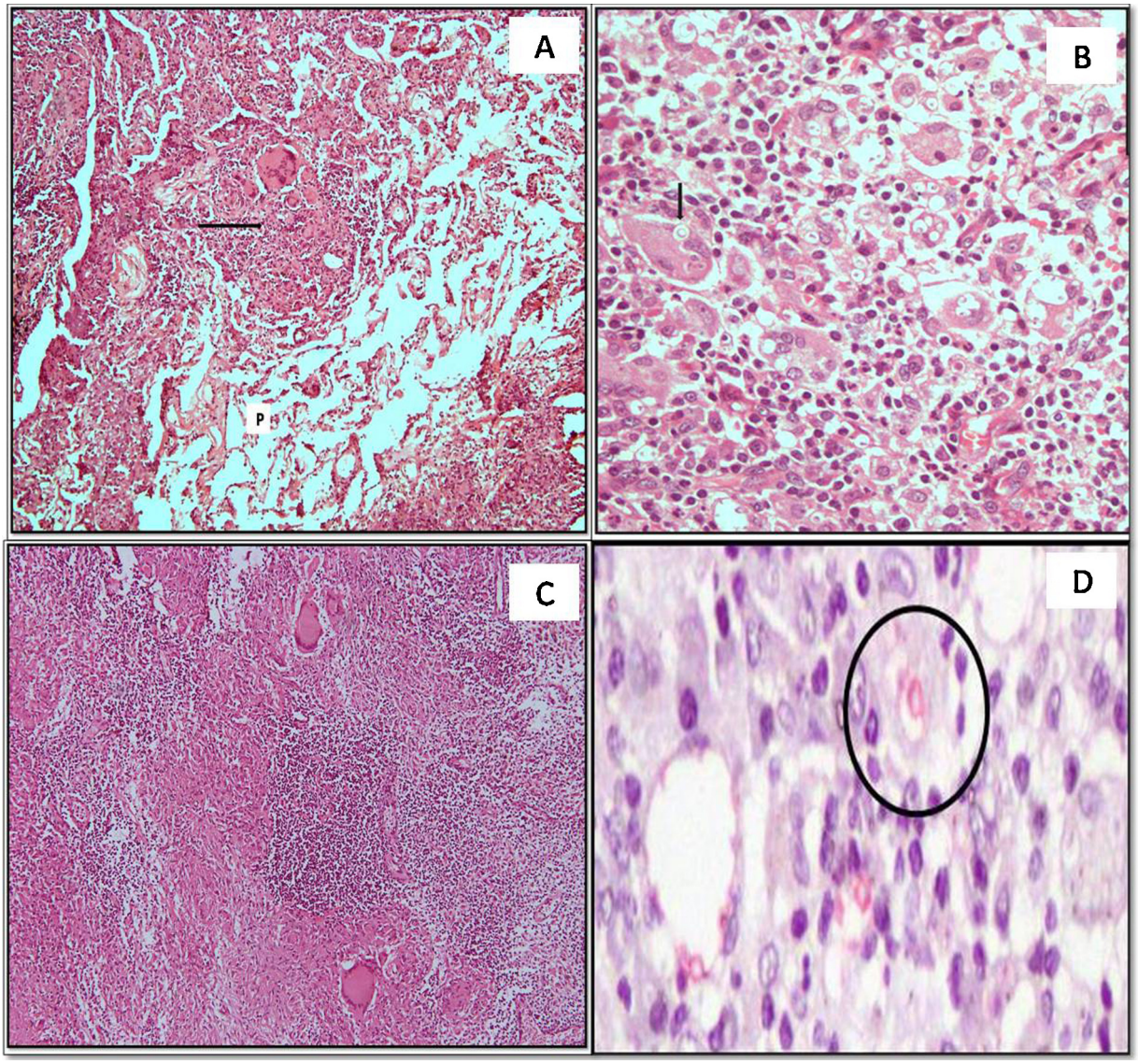

Fig. 2 – (A) hematoxylin and eosin (H&E 20×) – granulomatous inflammation in the lung parenchyma (P). (B) (H&E 40×) – numerous yeast forms of fungus both in intra- as well as extracellular locations. (C) (H&E 20×) – granulomatous inflammation in the lymph nodes. (D) The fungal capsule staining positive with mucicarmine stain.

Cryptococcosis is usually caused by one of two species namely *Cryptococcus neoformans*, which is a casual agent in majority of the cases, and *Cryptococcus gattii*, which is responsible for a smaller proportion of cases, often in an immuno-competent host. The portal of entry is the lung, with the acquisition of organism by inhalation of the soil-inhabiting yeast forms, and thus beginning initially as pulmonary disease. Other major sites involved include brain/meninges, lymph nodes and bones with the central nervous system being the most common site.

Clinically and radiologically, wide variety of features is described. Clinical presentation ranges from asymptomatic pulmonary colonisation to life-threatening meningitis and disseminated infection. Clinically, two forms predominate – pulmonary and cerebro-meningeal cryptococcosis, resulting from haematogenous dissemination from a pulmonary focus. Dissemination from a pulmonary infection less often results in cutaneous, muco-cutaneous, osseous and visceral forms. In disseminated infection, primary in lung is frequently undetected. The mortality rate is high in such cases of disseminated disease. Presenting symptoms commonly include fever, cough, weight loss, dyspnoea and chest pain. However, in about 30% cases, the disease may be entirely asymptomatic and usually detected incidentally. Haemoptysis is uncommon in immuno-competent patients.[2] On radioimaging studies, the most common finding in cryptococcosis is presence of single or multiple nodules usually in sub-pleural location ranging from 0.5 to 3 cm in diameter. As compared to other mycotic infections, cavitations are relatively uncommon. Other radiological abnormalities described include diffuse or focal interstitial opacities, alveolar opacities, cavitary lesions, pleural effusions and adenopathies. Immuno-competent

patients frequently show single or multiple peripheral nodules usually without cavitation as compared to immuno-compromised patients in whom cavitations are more common. In addition, pleural effusion and lymphadenopathies are more frequent findings in immuno-compromised patients.[3] Radiological and clinical findings may mimic a neoplastic process. The definitive diagnosis relies on demonstration of organisms in lung biopsy, bronchoalveolar lavage or fine-needle aspirate specimens. *C. neoformans* morphologically is a capsulated fungus measuring 5–10 μm in diameter having a thick capsule. On histopathology, there is a wide spectrum of inflammatory response varying from little or no inflammation to purely granulomatous reaction. At times, particularly in disseminated infection, the organisms multiply profusely with no apparent host response, whereas positive culture of CSF is definitive. Positive culture of respiratory secretions, especially in patients without clinical symptoms, needs additional supporting evidence. Serum cryptococcal antigen detection serves as an additional diagnostic tool. The recommended management of localised pulmonary cryptococcosis in an immuno-competent host is administration of oral fluconazole (200–400 mg per day) for 6–12 months. Itraconazole, voriconazole or posaconazole are other alternative drugs.[4]

Coexistence of tuberculosis and cryptococcus in lung has been described previously mostly in HIV patients or in immuno-suppressed patients. Its occurrence in an immuno-competent host is very rare with only isolated case reports. In most of these cases, the coexistent infections were diagnosed at different sites, usually central nervous system (CNS) or bone along with lung.[5] These patients come to light usually when they have disseminated disease. The natural history of pulmonary cryptococcal infection in immuno-suppressed patients is of dissemination and progression in the majority of the cases with high mortality whereas immuno-competent patients may present with more localised and self-limiting form. Huang et al. reported a rare case of tuberculous lymphadenopathy concomitant with pulmonary cryptococcus infection and highlighted the importance of testing for occult infection if the clinical response is insufficient.[6] This case is unique in that the coexistent cryptococcus and tubercular infections were diagnosed in an immuno-competent host and were localised to lung without CNS or any other site involvement. It has been suggested that both tuberculosis and cryptococcus have immuno-modulatory effects on host defences predisposing to second infection.[6] The rarity of this coexistence may have arisen because most of these cases occur in immuno-compromised patients with high mortality so the coexistent infections go undetected.

This case corroborates with the previous studies that cryptococcus can occur in healthy individuals. The presence of haemoptysis, lymphadenopathy and granulomatous inflammation on histopathology as seen in this case is more commonly associated with tuberculosis. Since the histomorphological features of both the infections are similar, it is suggested that in cases of pulmonary cryptococcosis in tissue specimens with marked granulomatous inflammation and associated lymphadenopathy, with or without haemoptysis, the possibility of this coexistent infection must be kept especially in endemic countries. A thorough search for AFB and tissue microbiological culture studies is warranted. Awareness of this coexistence in immuno-competent persons is important as it carries important diagnostic and therapeutic implications.

Conflicts of interest

The authors have none to declare.

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Case Report

Drug-resistant tuberculosis coexisting with invasive candidiasis in an immunocompetent 30-year-old woman: A case report

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ABSTRACT

Invasive candidiasis coexisting with drug-resistant *Mycobacterium tuberculosis* (DR-TB) in the immunocompetent patient is a rare entity. We report a 30-year-old woman, nondiabetic, who presented to us with complaints of acute onset cough, breathlessness, and fever since 20 days. On thorough investigations, she was diagnosed to be suffering from coexisting drug-resistant tuberculosis and invasive candidiasis. Prompt treatment initiated at right time helped us in saving her life. The unique presentation of this case and that too in an immunocompetent female makes it an interesting case.

1. Case history

A-30-year-old woman presented with complaints of right-sided chest pain, progressive shortness of breath, high grade fever, and dry cough of three weeks duration. She was a nonsmoker housewife and had no comorbid illness. She was diagnosed as pulmonary tuberculosis three to four years back for which she had taken incomplete and irregular anti-tubercular treatment.

On examination, the patient was tachypneic with respiratory rate of 35/min, hemodynamically stable, and febrile. Chest auscultation revealed decreased breath sounds over right hemithorax with hyper-resonant percussion note. Chest radiograph revealed right-sided pneumothorax along with underlying lung consolidation. Right intercostal tube (ICT) drainage was performed. However, there was no improvement in patient's distress. Post ICT chest X-ray revealed no expansion of lung (Fig. 1). There was presence of large bronchopleural fistula. In view of persistent respiratory distress and hypoxemia, she was electively intubated and mechanically ventilated. Hematological investigations revealed leukocytosis of 14,470 cells/μl with 95% neutrophils. Her renal and liver functions were normal. She was nonreactive for human immunodeficiency virus (HIV), and endotracheal aspirate (ETA) was negative for acid-fast bacilli and bacteria. The KOH wet mount of ETA showed budding yeast cells. 2D echocardiography revealed normal cardiac functions. Pleural fluid analysis revealed exudative fluid with lymphocytic predominance and adenosine deaminase levels of 37. The chest computed tomographic scan was done which showed diffuse ground glass opacity in right lung and left upper lobe with right pneumothorax with ICT in situ (Fig. 2). She was started on broad spectrum antibiotics but her symptoms worsened. Bronchoscopy was done and bronchoalveolar lavage was sent. Fungal culture revealed *Candida*

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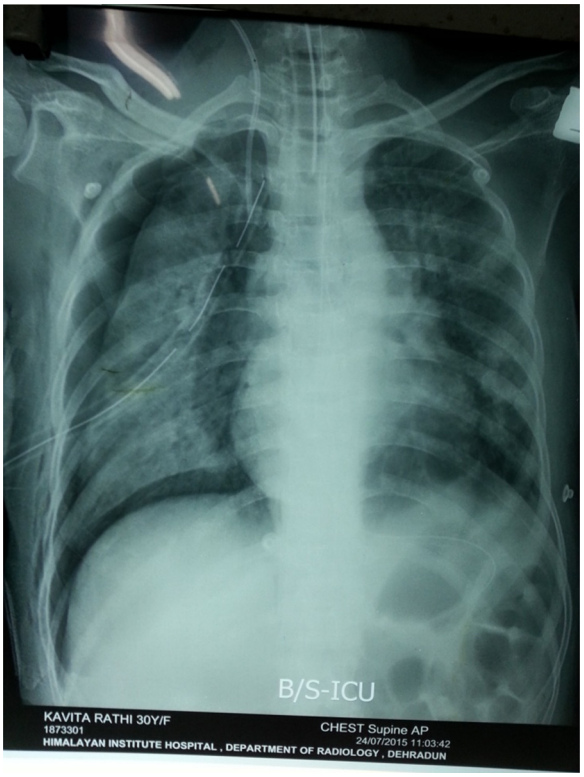

Fig. 1 – Chest X-ray of patient showing right-sided pneumothorax with underlying ground glassing with ICT in situ.

albicans sensitive to all antifungals. She was, hence, started on injection amphotericin B. She responded wonderfully to antifungal and could be weaned off from ventilator. However, her repeated chest X-ray showed no signs of improvement. Her pleural fluid was sent for drug sensitivity testing to rule out possibility of reactivation of tuberculosis using cartridge based nucleic acid amplification technique (CBNAAT). It turned out to be positive with presence of *Mycobacterium tuberculosis* resistant to rifampicin. She was started on treatment for multidrug-resistant TB (as per programmatic management of drug resistant tuberculosis)[1] and is responding well to the treatment. She is still in our follow-up.

2. Discussion

Drug-resistant tuberculosis (DR-TB) caused by *Mycobacterium tuberculosis* resistant to either isoniazid and rifampicin with or without resistance to other drugs has been an area of growing concern and is posing a threat to the control of tuberculosis (TB).[1] It is to be noticed that rifampicin resistance is quite rare without isoniazid resistance.[1] The great majority of DST results with rifampicin resistance will also be isoniazid resistance, that is, MDR-TB. Therefore, RNTCP has taken the programmatic decision that patients who have any Rifampicin resistance should also be managed as if they are a MDR-TB case, even if they do not formally qualify as an MDR-TB case.[1] The Global Tuberculosis Report 2014 estimated that 3.5% of

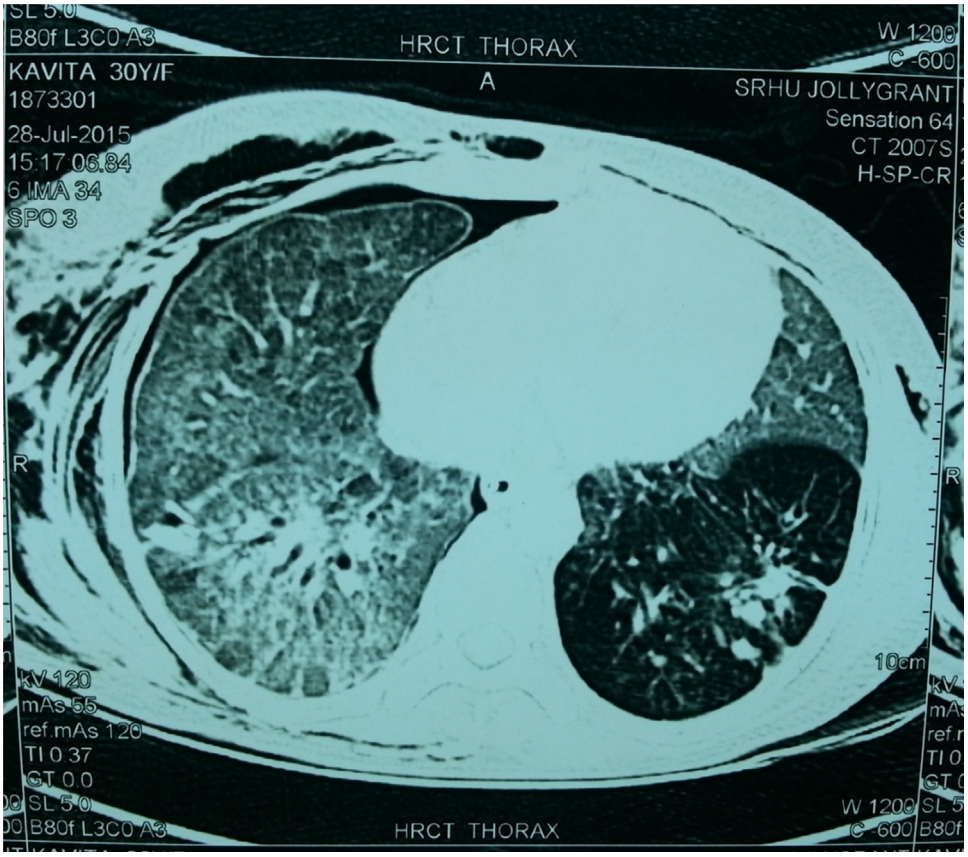

Fig. 2 – CECT thorax reveals diffuse ground glass opacity in right lung and left upper lobe with right pneumothorax with ICT in situ with subcutaneous emphysema.

newly diagnosed and 20.5% of previously treated TB cases had MDR-TB.[2] In India, estimates showed that the prevalence of MDR-TB among new and previously treated patients was 2.2% and 15%, respectively.[2] The MDR-TB is a human-made problem and results largely from poorly managed cases of TB.[3] While host genetic factors may probably contribute, incomplete and inadequate treatment is the most important factor leading to the development of MDR-TB.[3] Johnson et al.,[4] in a study of 109 culture-positive pulmonary tuberculosis patients, found a high incidence of drug resistance in previous treatment defaulters while only four of the 27 new incident cases had MDR-TB. The various reasons for default included travel to different places, symptom relief, adverse drug reactions, and inability to afford treatment.[4] In our patient also, irregular and inadequate treatment of tuberculosis led her to the development of MDR-TB.

Candidiasis is a primary or secondary mycotic infection caused by members of the genus *Candida*. The clinical manifestations may be acute, subacute, or chronic to episodic.[5] Involvement may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, or the gastrointestinal tract, or become systemic as in septicemia, endocarditis, and meningitis.[5] In healthy individuals, *Candida* infections are usually due to impaired epithelial barrier functions and occur in all age groups, but are most common in the newborn and the elderly. They usually remain superficial and respond readily to treatment. Systemic candidiasis is usually seen in patients with cell-mediated immune deficiency, and those receiving aggressive cancer treatment, immunosuppression, or transplantation therapy.[6] *Candida* spp. are considered opportunistic pathogens, even though they belong to the normal human microbiota.[7] A critical point for health professionals is to distinguish fungal colonization from fungal infection, especially in pulmonary samples.[7] Fungal infections and that too in invasive form usually occur in immunocompromised patients. Our patient was otherwise healthy three weeks before the disease and presented with short history which is against the usual presentation seen either with MDR-TB or invasive candidiasis. However, studies show that *M. tuberculosis* promoted down-modulatory immune mediators to counteract Th1-type cells and patients' innate immunity, and might have suppressive effects on the host's immune system[6,8] leading to invasive candidiasis in immunocompetent female.

Our patient became a diagnostic challenge because of various reasons. Her presentation was consistent with possible diagnosis of acute pyogenic pneumonia. However, there was no growth in sputum culture nor was there any improvement with high end broad spectrum antibiotics. Our patient also had clinico-radiological dissociation. Presence of diffuse ground glassing more in one lung with acute symptomatic presentation usually suggests either atypical pneumonia or acute interstitial pneumonia. Both tuberculosis and invasive candidiasis usually do not have this type of radiological presentation.

3. Conclusion

Tuberculosis and fungal infections can have atypical presentation both clinically and radiologically. They are usually kept low down in the differential list of causes leading to acute presentation in immunocompetent patients. However, they should be always kept in mind while treating such patients to prevent morbidity and mortality.

Conflicts of interest

The authors have none to declare.

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Letter to the Editor

Assessing worldwide research productivity on tuberculosis over a 40-year period: A bibliometric analysis

Tuberculosis (TB) remains a major public health problem globally, causing 10.4 million new cases and 1.9 million deaths worldwide in 2015.[1] The Global Tuberculosis Report, published by the World Health Organization (WHO) in 2016 stated that six countries accounted for 60% of the new cases: India, Indonesia, China, Nigeria, Pakistan and South Africa.[1] Despite the constant and slow decline in TB incidence, there are still a few challenges confronting the control of the infection, especially in developing countries, including the association between TB and poverty, the emergence of multidrug-resistant TB and the co-infection with HIV and other infectious diseases.[2,3]

Research on TB is a fundamental key in the process to eliminate this bacterial infection as a threat to global health and the analysis of the scientific output provides valuable information related to trends in time and the epidemiology of the disease. For that reason, assess the current status of global scientific productivity on TB is highly relevant. Therefore, we conducted a bibliometric study in two major databases: PubMed/Medline (via GoPubMed®) and Scopus during the period 1977–2017 (up to January 2017) according to the main operator "Tuberculosis" in the strategy search.

At Medline, a total of 223,861 documents were retrieved with an annual production of 5596 articles. Authors from 169

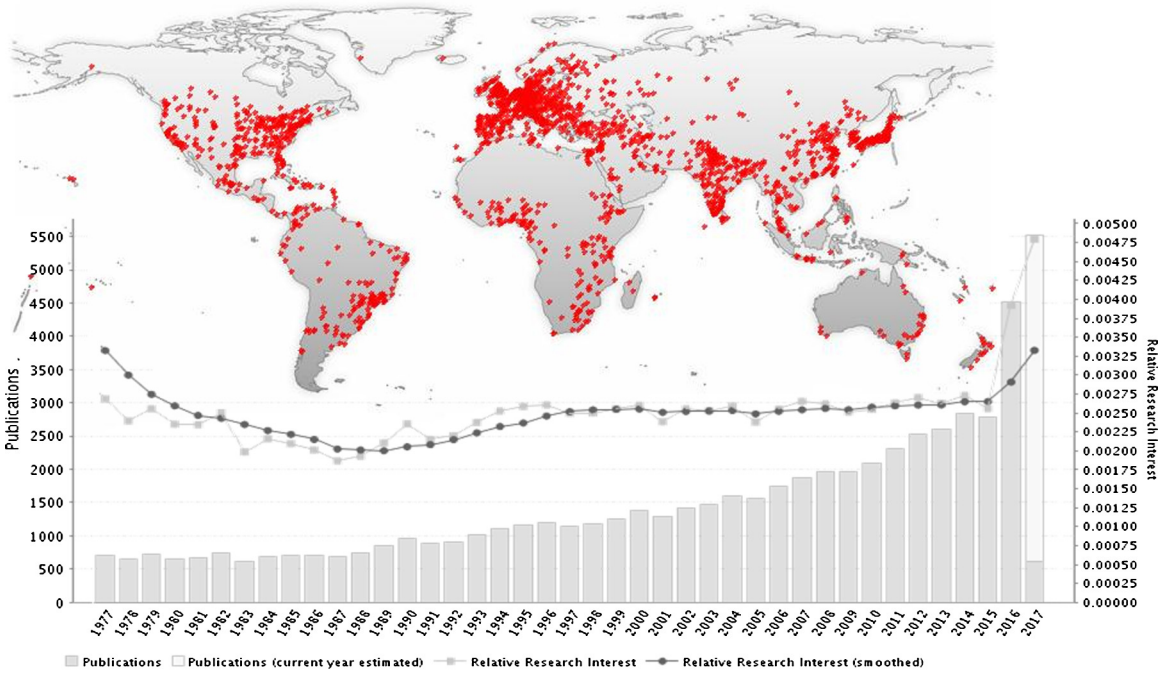

Fig. 1 – Worldwide scientific production related to tuberculosis by country, number of publications and trends in time, 1977–2017 (using GoPubMed®).

different countries contributed to retrieved documents. USA ranked first in the number of publications with the 4.33% of total, followed by India (1.68%), United Kingdom (1.33%), China (1.21%) and Japan (1.12%). In contrast, higher scientific production was observed in Western Europe (6.01%) than the Asian and African countries (such as Indonesia, Nigeria and Pakistan), where TB is highly prevalent and lethal. Trends in time (Fig. 1) clearly reveal a rise of the TB-related publications over the years with a peak of production in 2016. The Scopus search retrieved 285,660 publications (14.19% from the USA, 6.67% from United Kingdom, 5.90% from India, 3.41% from Japan and 3.23% from France) with an annual production of 7.141 articles with an increasing of publications in the last 20 years. The themes in the articles were varied, ranging from immunology, molecular biology, diagnostic tools, epidemiology, co-infections, comorbidities and treatment options.

These results are consistent with a previous bibliometric study of TB research in PubMed published in 2008, where the USA was the leader, followed by India, Japan and the United Kingdom.[4] However, the average annual growth rate in this study was higher, which translate into an increased interest in TB research over the last years, in contrast with the low scientific productivity related to other emerging infectious diseases.[5]

In conclusion, the amount of literature related to TB research has considerably increased over the last 40 years. This bibliometric analysis has demonstrated the leading role that the USA, India, UK, China and Japan play in TB research. The most affected countries produced fewer publications than other world countries. Thus, more research and international efforts are urgently needed in these low resource countries to stop and reverse TB worldwide.

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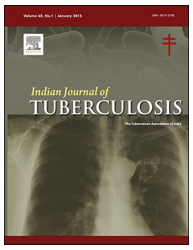

Abstracts

Novel interferon-gamma assays for diagnosing tuberculosis in young children in India

Shaikh N, Gupte A, Dharmshale S, et al. *Int J Tuberc Lung Dis*. 2017;21(4):412–419. http://dx.doi.org/10.5588/ijtld.16.0428.

Setting: The tuberculin skin test (TST) and interferon-gamma release assays (IGRAs) are used as supportive evidence to diagnose active tuberculosis (TB). Novel IGRAs could improve diagnosis, but data are lacking in young children.

Design: Children (age ≤5 years) with suspected TB were prospectively screened at a tertiary hospital in Pune, India; the children underwent TST, and standard (early secretory antigenic target 6 and culture filtrate protein 10) and enhanced (five additional novel antigens) enzyme-linked immunospot (ELISpot) assays.

Results: Of 313 children (median age 30 months) enrolled, 92% had received bacille Calmette–Guérin vaccination, 53% were malnourished and 9% were coinfected with the human immunodeficiency virus (HIV); 48 (15%) had TB, 128 (41%) did not, and TB could not be ruled out in 137 (44%). The sensitivity of enhanced (45%) and standard (42%) ELISpot assays for diagnosing TB was better than that of TST (20%) (P □ 0.03); however, enhanced ELISpot was not more sensitive than the standard ELISpot assay ($P = 0.50$). The specificity of enhanced ELISpot, standard ELISpot and TST was respectively 82% (95% CI 74–89), 88% (95% CI 81–94) and 98% (95% CI 93–100). Rv3879c and Rv3615c, previously reported to be promising antigens, failed to improve the diagnostic performance of the ELISpot assay.

Conclusion: The TST and the standard and novel ELISpot assays performed poorly in diagnosing active TB among young children in India.

Predictors of unfavourable treatment outcome in patients with multidrug-resistant tuberculosis in India

Nair D, Velayutham B, Kannan T, et al. *Public Health Action*. 2017;7(1):32–38. http://dx.doi.org/10.5588/pha.16.0055.

Setting: India has one of the highest global rates of multidrug-resistant tuberculosis (MDR-TB), which is associated with poor treatment outcomes. A better understanding of the risk factors for unfavourable outcomes is needed.

Objectives: To describe (1) the demographic and clinical characteristics of MDR-TB patients registered in three states of India during 2009–2011, (2) treatment outcomes, and (3) factors associated with unfavourable outcomes.

Design: A retrospective cohort study involving a record review of registered MDR-TB patients.

Results: Of 788 patients, 68% were male, 70% were aged 15–44 years, 90% had failed previous anti-tuberculosis treatment or were retreatment smear-positive, 60% had a body mass index <18.5 kg/m^2 and 72% had additional resistance to streptomycin and/or ethambutol. The median time from sputum collection to the start of MDR-TB treatment was 128 days (IQR 103–173). Unfavourable outcomes occurred in 40% of the patients, mostly from death or loss to follow-up. Factors significantly associated with unfavourable outcomes included male sex, age □ 45 years, being underweight and infection with the human immunodeficiency virus. Adverse drug reactions were reported in 24% of patients, with gastrointestinal disturbance, psychiatric morbidity and ototoxicity the most common.

Conclusion: Long delays from sputum collection to treatment initiation using conventional methods, along with poor treatment outcomes, suggest the need to scale up rapid diagnostic tests and shorter regimens for MDR-TB.

MSMEG_5684 down-regulation in *Mycobacterium smegmatis* affects its permeability, survival under stress and persistence

Keshari D, Singh KS, Sharma R, Yadav S, Singh SK. *Tuberculosis*. 2017;103:61–70. http://dx.doi.org/10.1016/j.tube.2017.01.004.

The *Mycobacterium tuberculosis* (*Mtb*) genome sequence and annotation details have been available for a long time; however physiological relevance of many ORFs remains poorly

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0019-5707/

described. *Mtb* is a pathogenic strain; hence, surrogate strains such as *Mycobacterium bovis* BCG and *Mycobacterium smegmatis* (*Msmeg*) have also been studied to gain an understanding of mycobacterial physiology and metabolism. The *Mycobacterium smegmatis* mc^2 155 ORF MSMEG_5684 is annotated as a part of serine biosynthetic pathway, however, its physiological significance remains to be established experimentally. To understand the relevance of SerC for *Msmeg* physiology we developed a recombinant *M. smegmatis* with SerC knockdown (KD) and also complemented it with *serC* over-expressing construct (KDC). The KD showed reduced growth compared to wild-type (WT) and complemented strain on glycerol as carbon source. The growth of KD was restored after supplementation of serine. The survival studies with WT and KD under oxidative, nitrosative and detergent stresses showed increased susceptibility of KD. The KD also showed increased susceptibility to antimycobacterial agents and poor ability for *in vitro* persistence. Also, the *serC* transcript profiling showed increased expression under stress. The complementation studies with *Msmeg serC* showed growth restoration of *Escherichia coli*-Δ*serC* in minimal medium.

A comparative study of drug susceptibility testing techniques for identification of drug resistant TB in a Tertiary Care Centre, South India

Anto Jesuraj Uday Kumar J, Srinivasa H, Chiramal JA. *J Tuberc Res*. 2017;5(1):44–57. http://dx.doi.org/10.4236/jtr.2017.51005.

India tops the global list for drug resistant tuberculosis, but inadequate and expensive laboratory culture techniques have led to delay in the diagnosis and treatment. We studied the potential of an alternative method which could be cost-effective by combining the drugs in the same tube for identification of drug resistance. Drug susceptibility test (DST) results of 1000 sputum samples are got from suspected TB patients against INH (isoniazid) and rifampicin by two techniques: (a) a modified technique with both drugs in the same MGIT tube and (b) a standard technique with the antibiotics in separate MGIT tubes for the diagnosis of MDR-TB (multidrug resistant). 39 samples were contaminated and were excluded from final analysis. 198 were smear positives by the concentrated Ziehl–Neelsen's staining method. 219 were found to be culture positive out of which 195 were identified as *Mycobacterium tuberculosis* complex. 40 (20.5%) strains were identified as MDR-TB by the conventional method and 39 were picked up by the modified DST. INH and rifampicin mono-resistance accounted for 32 (16.4%) and 4 (2%) respectively. 99% concordance was observed between the two tests in categorizing MDR-TB. Similarly modified technique with combination of the second line Antibiotics-Ofloxacin, Kanamycin and Capreomycin was applied on the identified MDR strains in a stepwise manner. 6 (15%) were identified as pre-XDR strains and 2 (5%) were found to be XDR-TB strains. This study implies that combining drugs in the same tube may be an equivalent and possibly a cost-effective alternative which needs to be explored further.

Use of cost effective semi-automated (manual/micro) MGIT system over BACTEC 960 to perform first line anti-tuberculosis drugs sensitivity testing

Mistry Y, Rajdev S, Mullan S. *J Tuberc Res*. 2017;5(1):227–234. http://dx.doi.org/10.4236/jtr.2016.44025.

Introduction: Multi-drug resistant tuberculosis (MDR-TB) that is the tuberculosis that is resistant to at least 2 of the first line anti-tuberculosis drugs is fatal infectious disease. Cases of MDR-TB are now increasing with 30,000 cases of MDR-TB reported in 2013 by national TB programme. Rapid diagnosis of MDR-TB is extremely important for rapid treatment of patient and to prevent spread of MDR-TB to other. BACTEC 960 system helps in rapid diagnosis but purchase of expensive instrument for the same is the limitation. However, the same purpose can be solved by use of semi-automated MGIT system.

Aims and objectives: Aim of this study is to do drug sensitivity testing of the first line anti-tuberculosis drugs with the use of semi-automated MGIT systems. 350 newly registered and suspected cases of tuberculosis in tertiary care hospital were included. Samples were processed for digestion and decontamination and inoculated in MGIT tubes and also on LJ medium. Reading was taken using semi-automated MGIT system. Positive tubes were confirmed by rapid test for *Mycobacterium tuberculosis* and then drug sensitivity was performed.

Result: Out of 350 samples, 62% were sputum; 33% were pleural fluid and rest 5% were lymph node, ascetic fluid, CSF, pus. Average day of positivity by MGIT was 13–20 days as compared to 25–37 days by solid medium, which was statistically significant with p value <0.01. MDR cases were 2% out of 350 samples.

Conclusion: Manual MGIT System is a simple, efficient, safe to use diagnostic system. It does not require any expensive/special instrumentation other than the UV lamp for detection of fluorescence. The rapidity by which mycobacteria are detected is the most important advantage of the Manual MGIT. In areas with limited resources where purchase of expensive instruments such as the MGIT960 is out of scope, the use of manual MGIT for rapid susceptibility testing for MDR-TB could be a possibility.

Safety and efficacy of the C-Tb skin test to diagnose *Mycobacterium tuberculosis* infection, compared with an interferon γ release assay and the tuberculin skin test: A phase 3, double-blind, randomised, controlled trial

Ruhwald M, Aggerbeck H, Gallardo RV, et al. *Lancet Respir Med*. 2017;5(4):259–268. http://dx.doi.org/10.1016/S2213-2600(16)30436-2.

Background: Targeted screening and treatment of *Mycobacterium tuberculosis* infection substantially reduces the risk of developing active tuberculosis. C-Tb (Statens Serum Institute, Copenhagen, Denmark) is a novel specific skin test based on ESAT-6 and CFP10 antigens. We investigated the safety and diagnostic potential of C-Tb compared with established tests in the contact-tracing setting.

Methods: Negative controls, close contacts, occasional contacts, and patients with active pulmonary tuberculosis were enrolled at 13 centres in Spain. We compared C-Tb with the QuantiFERON-TB Gold In-Tube ([QFT] Qiagen, Hilden, Germany) interferon γ release assay (IGRA) and the purified protein derivative (PPD) RT 23 tuberculin skin test ([TST] Statens Serum Institute). All participants older than 5 years were tested with QFT. Some participants in the negative control group received C-Tb without the TST to test for potential interactions between C-Tb and PPD RT 23. The rest were randomly assigned in blocks of ten and tested with both C-Tb and TST, with five in each block receiving injection of C-Tb in the right arm and the TST in the left arm and five vice versa. The primary and safety analyses were done in all participants randomly assigned to a group who received any test. This trial is registered with ClinicalTrials.gov, number NCT01631266, and with EudraCT, number 2011-005617-36.

Findings: From July 24, 2012, to October 2, 2014, 979 participants were enrolled, of whom 263 were negative controls, 299 were occasional contacts, 316 were close contacts, and 101 were patients with tuberculosis. 970 (99%) participants completed the trial. Induration sizes were similar for C-Tb and TST, but TST positivity was affected by BCG vaccination status. We found a strong positive trend towards C-Tb test positivity with increasing risk of infection, from 3% in negative controls to 16% in occasional contacts, to 43% in close contacts. C-Tb and QFT results were concordant in 785 (94%) of 834 participants aged 5 years and older, and results did not differ significantly between exposure groups. The safety profile of C-Tb was similar to that for the TST.

Interpretation: C-Tb delivered IGRA-like results in a field-friendly format. Being unaffected by BCG vaccination status, the C-Tb skin test might provide more accurate treatment guidance in settings where the TST is commonly used.

Funding: Statens Serum Institute.

The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis

Dheda K, Gumbo T, Maartens G, et al. *Lancet Respir Med*. 2017;5 (4):291–360. http://dx.doi.org/10.1016/S2213-2600(17)30079-6.

Global tuberculosis incidence has declined marginally over the past decade, and tuberculosis remains out of control in several parts of the world including Africa and Asia. Although tuberculosis control has been effective in some regions of the world, these gains are threatened by the increasing burden of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis. XDR tuberculosis has evolved in several tuberculosis-endemic countries to drug-incurable or programmatically incurable tuberculosis (totally drug-resistant tuberculosis). This poses several challenges similar to those encountered in the pre-chemotherapy era, including the inability to cure tuberculosis, high mortality, and the need for alternative methods to prevent disease transmission. This phenomenon mirrors the worldwide increase in antimicrobial resistance and the emergence of other MDR pathogens, such as malaria, HIV, and Gram-negative bacteria. MDR and XDR tuberculosis are associated with high morbidity and substantial mortality, are a threat to health-care workers, prohibitively expensive to treat, and are therefore a serious public health problem. In this Commission, we examine several aspects of drug-resistant tuberculosis. The traditional view that acquired resistance to antituberculous drugs is driven by poor compliance and programmatic failure is now being questioned, and several lines of evidence suggest that alternative mechanisms—including pharmacokinetic variability, induction of efflux pumps that transport the drug out of cells, and suboptimal drug penetration into tuberculosis lesions—are likely crucial to the pathogenesis of drug-resistant tuberculosis. These factors have implications for the design of new interventions, drug delivery and dosing mechanisms, and public health policy. We discuss epidemiology and transmission dynamics, including new insights into the fundamental biology of transmission, and we review the utility of newer diagnostic tools, including molecular tests and next-generation whole-genome sequencing, and their potential for clinical effectiveness. Relevant research priorities are highlighted, including optimal medical and surgical management, the role of newer and repurposed drugs (including bedaquiline, delamanid, and linezolid), pharmacokinetic and pharmacodynamic considerations, preventive strategies (such as prophylaxis in MDR and XDR contacts), palliative and patient-orientated care aspects, and medicolegal and ethical issues.

Serial QuantiFERON testing and tuberculosis disease risk among young children: An observational cohort study

Andrews JR, Nemes E, Tameris M, et al. *Lancet Respir Med*. 2017;5 (4):282–290. http://dx.doi.org/10.1016/S2213-2600(17)30060-7.

Background: The value of quantitative interferon-γ release assay results for predicting progression from *Mycobacterium tuberculosis* infection to active disease is unknown. We aimed to investigate the relation between QuantiFERON-TB Gold In-Tube (QFT) conversion interferon-γ values and risk of subsequent active tuberculosis disease and of QFT reversion.

Methods: We analysed data from a reported vaccine efficacy trial of the tuberculosis vaccine MVA85A in South Africa. QFT negative, HIV uninfected young children aged 18–24 weeks were enrolled. We stratified participants by quantitative QFT result (interferon-γ < 0.35 IU/mL, 0.35–4.00 IU/mL, and >4.00 IU/mL) at the intermediate study visit (day 336) and determined risk of progression to active tuberculosis disease over the subsequent 6–24 months. No QFT differences were observed between placebo and MVA85A groups at day 336 or end of study; therefore, both groups were included in analyses. Study clinicians were not masked to QFT values, but strict case definitions were used that excluded QFT results. We used generalised additive models to evaluate the quantitative relation between day 336 QFT value and subsequent disease risk, and we compared disease rates between QFT strata using a two-sample Poisson test.

Findings: Among 2512 young children with QFT tests done at day 336, 172 (7%) were positive; 87 (7%) of 1267 in placebo

group and 85 (7%) of 1245 in the MVA85A group ($p = 1.00$). Compared with QFT non-converters (tuberculosis disease incidence 0.7 per 100 person-years [95% CI 0.4–1.1]), children with QFT conversion at interferon-γ values between 0.35 and 4.00 IU/mL did not have significantly increased risk of disease (2.5 per 100 person-years [95% CI 0.4–9.4]; incidence rate ratio (IRR) 3.7 (95% CI 0.4–15.8; $p = 0.23$). However, QFT conversion at interferon-γ values higher than 4.00 IU/mL was associated with substantially increased disease incidence (28.0 per 100 person-years [95% CI 14.9–45.7]) compared with non-converters (IRR 42.5 [95% CI 17.2–99.7]; $p < 0.0001$), and compared with children with interferon-γ values between 0.35 and 4.00 IU/mL (IRR 11.4 [95% CI 2.4–107.2]; $p = 0.00047$). Among 91 QFT converters who were given a repeat test, 53 (58%) reverted from positive to negative. QFT reversion risk was inversely associated with interferon-γ value at QFT conversion and was highest with interferon-γ values less than 4.00 IU/mL (47 [77%] of 61).

Interpretation: In young children, tuberculosis disease risk was not significantly increased, and QFT reversion was common, following QFT conversion at interferon-γ values up to 10 times the recommended test threshold (0.35 IU/mL). By contrast, QFT conversion at very high interferon-γ values (>4.00 IU/mL) warrants intensified diagnostic and preventive intervention because of the extremely high risk of tuberculosis disease in these young children.

Funding: Aeras, Wellcome Trust, and Oxford-Emergent Tuberculosis Consortium (OETC) were the funders of the MVA85A 020 Trial. National Institute of Allergy and Infectious Diseases supported this analysis.

Effect of new tuberculosis diagnostic technologies on community-based intensified case finding: A multicentre randomised controlled trial

Calligaro GL, Zijenah LS, Peter JG, et al. *Lancet Infect Dis*. 2017;17 (4):441–450. http://dx.doi.org/10.1016/S1473-3099(16)30384-X.

Background: Inadequate case detection results in high levels of undiagnosed tuberculosis in sub-Saharan Africa. Data for the effect of new diagnostic tools when used for community-based intensified case finding are not available, so we investigated whether the use of sputum Xpert-MTB/RIF and the Determine TB LAM urine test in two African communities could be effective.

Methods: In a pragmatic, randomised, parallel-group trial with individual randomisation stratified by country, we compared sputum Xpert-MTB/RIF, and if HIV-infected, the Determine TB LAM urine test (novel diagnostic group), with laboratory-based sputum smear microscopy (routine diagnostic group) for intensified case finding in communities with high tuberculosis and HIV prevalence in Cape Town, South Africa, and Harare, Zimbabwe. Participants were randomly assigned (1:1) to these groups with computer-generated allocation lists, using culture as the reference standard. In Cape Town, participants were randomised and tested at an Xpert-equipped mobile van, while in Harare, participants were driven to a local clinic where the same diagnostic tests were done. The primary endpoint was the proportion of culture-positive tuberculosis cases initiating tuberculosis treatment in each study group at 60 days. This trial is registered at ClinicalTrials.gov, number NCT01990274.

Findings: Between October 18, 2013, and March 31, 2015, 2261 individuals were screened and 875 (39%) of these met the criteria for diagnostic testing. 439 participants were randomly assigned to the novel group and 436 to the routine group. 74 (9%) of 875 participants had confirmed tuberculosis. If late culture-based treatment initiation was excluded, more patients with culture-positive tuberculosis were initiated on treatment in the novel group at 60 days (36 [86%] of 42 in the novel group *vs* 18 [56%] of 32 in the routine group). Thus the difference in the proportion initiating treatment between groups was 29% (95% CI 9–50, $p = 0.0047$) and 53% more patients initiated therapy in the novel diagnostic group than in the routine diagnostic group. One culture-positive patient was treated based only on a positive LAM test.

Interpretation: Compared with traditional tools, Xpert-MTB/RIF for community-based intensified case finding in HIV and tuberculosis-endemic settings increased the proportion of patients initiating treatment. By contrast, urine LAM testing was not found to be useful for intensive case finding in this setting.

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