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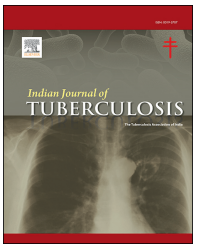

Editorial

Covid-19 vaccines and new mutant strains impacting the pandemic

Vaccines are the key for preventing the zoonotic virus pandemic caused by SARS-Cov-2 with no specific antidote. Various countries are working to roll out the vaccines.[1,2] There are four categories of vaccines in clinical trials namely; *WHOLE VIRUS, PROTEIN SUBUNIT, VIRAL VECTOR and NUCLEIC ACID (RNA AND DNA)*. Some of them try to induce the antigen into the body, while others use the body's own cells to make the viral antigen. Till February 2021, at least seven different vaccines across three different platforms have been rolled out in various countries. Around 100 plus vaccine candidates are in development in different phases.[3]

Three new clinically important mutant variants of the virus, taking the world by storm have been identified as:

- The United Kingdom: Identified a London variant called B.1.1.7 (variant VUI-202012/01) with 23 mutations, many of them associated with alterations in a protein made by the virus in the spike protein. Compared to other variants, it spreads more easily and quickly.[4,5] Experts reported (January 2021) that it may be associated with an increased risk of death compared to other variant viruses, but more studies are needed to confirm this finding. It has been detected in many countries.
- South Africa: A variant called B.1.351 emerged independently of B.1.1.7. Detected in early October 2020, B.1.351 shares some mutations with B.1.1.7. Many cases were reported from US in January 2021.
- Brazil: A variant called P.1 emerged among travellers from Brazil, who were tested during routine screening at an airport in Japan, in early January. Variant contains an additional set of mutations that may affect its ability to be recognized by antibodies. This variant was detected in the US in January 2021.

These new variants are capable of spreading faster are emerging and leading to inevitable questions about whether they will make the newly approved vaccines less effective. PCR is the main diagnostic tool which targets 3 parts of the virus to confirm presence of an infection. As per researchers, one target showed repeated negative samples from some parts in England with rapidly rising case numbers, while other two showed positive results. Scientists found that the virus in these samples had picked up a mutation – a deletion at the same H69 and V70 positions in the protein meaning that PCR was unable to pick the result in some cases. A new lineage of the Covid-19 virus was discovered that had picked up multiple mutations over a relatively short period of time. They designated B117 – the new British Covid-19 variant, also known as VOC 202012/01 which has spread to 50 other countries by mid-January. It is estimated to be 50–75% more transmissible than the original Covid-19 virus.

1. Other new strains

Other new strains of significance spreading in the US were 20C-US and the CAL.20C in California. There are reports of this variant infecting people who have already been infected with a different variant of the virus last year. One of the mutations carried by CAL.20C, (L452R,) found on the spike protein is being associated with a decreased sensitivity to antibodies, which suggests that it may be able to evade parts of the immune system. Another variant (B1.526) is circulating in New York in February 2021 has also worried scientists. By mid-February, it accounted for 12.3% of the viruses analysed. It contains two E484K and N501Y, which was also present in variants from Brazil and South Africa. In most cases, treatments and vaccines causes the viruses to evolve and continue spread. Those viruses that resist treatment survive for longer to replicate and spread their genetic material. Hence according to researchers, efforts to spot new variants of Covid-19 as early as possible should include testing sewage samples in cities like Belo Horizonte in Brazil. Hence genome sequencing is the need of the hour.

Another new variant has been located in Finland (Fin-796H) and is reported to have mutations similar to those seen in B.1.1.7 and B.1.351. It also has a mutation in one of the regions (N) recognized by PCR testing. But as per experts, that should not cause a major problem as PCR relies on 2/3 different assays that detect different parts of the virus. Similar issue was seen with in B.1.1.7 variant which escaped the assay that detects the S gene of the virus.[6,7]

2. In future, corona virus may evade vaccines/treatments

Slow vaccine rollout, mutated strain which is 50% more contagious, may undermine diagnostic testing, antibody treatment and vaccine efficacy. At present the 2 Covid-19 vaccines for use in the US remain highly effective for now. A high level of immunity appears to last for 8 months or more as per research. Researchers around the world agree that the threat of vaccine evading mutations are possible though not necessarily imminent but all also agree that urgent action is required to prevent such a situation. Hence solution is to double down on public health measures to quickly roll out the vaccines.

3. Increased transmission can increase mutations

The mutated variant that originated in the U.K., called B.1.1.7 allows the spikes on the virus to bind easily with receptors on human cells. B-117 is not a deadly virus, but because of its high infectivity it kills more people than the original strain would have. B-117 has the capability to grow in communities where SARS-CoV-2 is kept under control. Till date the two most notable mutated strains B-117 and 501. V2, from South Africa do not seem to sidestep vaccines or natural immunity. Pfizer announced that a preliminary study finds its vaccine holds up against the B-117 mutations, noting that the nature of the vaccine's construction should allow adjustments to meet the challenge of corona virus mutations.

4. Slow progress

One of the major reasons which can give the virus time to mutate its spike protein and escape the vaccine in some countries is the slow vaccine rollout. As per scientists, some vaccines could even start to exacerbate Covid-19 infections via a phenomenon known as antibody dependent enhancement, where certain antibodies end up contributing to the severity of the disease. Vaccine producers like Moderna is developing a booster dose to better tackle emerging SARS-CoV-2 strains. AstraZeneca quoted that in 6–9 months times they may come out with a vaccine for new strains. German biotech Cure Vac recently has collaborated with GlaxoSmithKline to develop messenger RNA (mRNA) vaccines.

The new generation of vaccines for Covid-19 are being given all over the world, but the new strains are a big challenge for them. As per reports, the Oxford vaccine is less effective against a variant common in South Africa than against other strains, causing the country to halt the distribution of that vaccine. A study assessing Israel's vaccination[8] showed that 2 doses of Prizer–Bio Tech vaccine reduces symptomatic cases by 94%, hospital admission by 87% and severe Covid-19 by 92%.As per the article the vaccine was also effective against B.1.1.7. Single dose Covid-19 vaccine made by Johnson and Johnson has been given emergency use authorization US FDA.[9] Based on analysis, data on 39,321 adults with no previous signs of infection reported the efficacy as 66.1% for preventing moderate to severe Covid-19, 28 days after vaccination. The company initially reported that the vaccine provided 72% protection against moderate to severe Covid-19 but proportion fell to 66% in Latin America and 57% in South Africa. There is no evidence suggesting vaccines will be any less effective against the new variant. As per experts they are not seeing any increased virulence spike protein, that may reduce vaccine effectiveness so Covid-19 vaccines appears to be adequate in generating an immune response to the variant of the corona virus.

5. Redesigning of Covid-19 vaccines

The immediate way to combat the threat of emerging variants is probably to quickly vaccinate as many people as possible with current shots. Researchers are of the opinion that new updated vaccines may be required in the future. South African variant called 501Y.V2 (also known as variant B.1.351), is of concern since it carries mutations that sap the potency of virus-inactivating 'neutralizing antibodies' that were made by people who received either the Pfizer/Moderna RNA vaccines. It is not clear if these changes are enough to lower the effectiveness of those vaccines. Other immune responses that vaccines prompt may help to protect against the effects of variants. Data released by Novavax concluded that experimental vaccine, designed to combat the original virus, was 85% effective against a United Kingdom and 50% effective against 501Y.V2. Hence drop in vaccines effectiveness is of high concern. According to the year, the flu vaccine has to be updated as the influenza virus mutates and adapts to escape the immunity already present in the population. If the corona virus shows similar capabilities, the vaccines may have to regularly updated. Vaccines like Moderna, Pfizer and AstraZeneca instruct cells to produce the virus's spike protein. Variants including 501Y.V2 carry spike mutations that alter regions targeted by neutralizing antibodies. Another possibility is to change older versions identified in Wuhan, China for an updated molecule with specific amino-acid changes that hinder antibody responses. It also needs to be determined whether any such changes would have knock-on effects that alter how the immune system reacts to the vaccine. Other options is to include both new and old forms of the spike protein in a single jab (multivalent vaccine).

6. Universal Covid-19 vaccine

Solution is to develop a universal vaccine that is future-proof against the evolving corona virus. Clue lies in examining vaccines for other viral infections that have stayed effective for decades. Yellow fever has a weakened form of yellow fever virus. Just like Covid-19, yellow fever is caused by an RNA virus. As per research, T cells largely ignore the surface antigens of the yellow fever virus. Instead, they recognize antigens within the virus. Influenza viruses mutate faster than Corona virus. They have a step in their replication process that proofreads the copied genetic code for errors, slowing the introduction of mutations. All depends on how quickly we can

get the pandemic under control. The likelihood of mutations depends on how much virus is circulating,[10,11] It is uncertain, how long immunity from the Covid-19 vaccine will last. A flu vaccine is needed every year not only because the flu virus mutates quickly but also because the antibody response wanes over time.[12] The present Covid-19 vaccines are expected to provide some protection against new virus variants, because they elicit a broad immune response. Hence changes/mutations in the virus should not make vaccines completely ineffective. If these vaccines prove to be less effective against one or more variants, it will be possible to change the composition of the vaccines to protect against these variants hence, in future vaccines may need to incorporate more than one strain when in development or booster shots may be required. Stopping Measures to reduce transmission including frequent hand washing, wearing a mask, physical distancing, good ventilation and avoiding crowded places or closed settings continue to work against new variants by reducing viral transmission thereby also reducing opportunities for the virus to mutate. High-risk groups have to be prioritized to maximize global protection against new variants and minimize the risk of transmission. Ensuring equitable access to Covid-19 vaccines is more critical than ever to address the evolving pandemic.

7. People's future response to revaccination with the updated vaccine

As per research, people tend to have more robust immune responses to the first variant of a pathogen that they encounter than subsequent variants. Hence updated vaccines may trigger lower immune responses as compared to the first one. As per evidence, RNA vaccines may not fall prey to this trend. But some RNA vaccines trigger surprisingly complex immune responses, yielding antibodies that target regions of viral proteins that are often not detected in responses to other kinds of vaccines which means that RNA vaccines will also be better able to target the changes present in a variant. Many US companies are planning to update their vaccines in the near future. A French company is working on an inactivated vaccine using the complete virus, to potentially form a response to all possible epitopes, a term for the portions of the virus's proteins that the immune system can recognize. It also combines the inactivated virus with an adjuvant. As more people get vaccinated, it is expected that the virus circulation should decrease, which will further lead to fewer mutations.

Conflicts of interest

The authors have none to declare.

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Shanker Matta, Epidemiologist
Sanjay Rajpal, Senior Chest Physician
K.K. Chopra, Director
New Delhi Tuberculosis Centre, India

V.K. Arora, Honorary Treasurer & Trustee*
TB Association of India, India

**Corresponding author.*
E-mail address: vijaykumar1945@gmail.com

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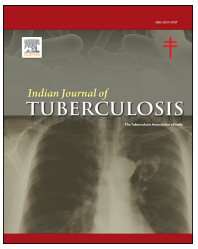

Original Article

Surgical treatment of tuberculous chronic constrictive pericarditis: A retrospective observational study from tertiary hospital of eastern Nepal

Lokesh Shekher Jaiswal, Dr., Associate professor[*],
Narendra Pandit, Dr., Associate professor,
Bijay Sah, Dr., Assistant Professor

Department of Surgery, B P Koirala Institute of Health Sciences (BPKIHS), Nepal

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ABSTRACT

Background: Tuberculosis remains an important cause of chronic constrictive pericarditis (CCP) in developing countries. It is a surgically treatable cause of diastolic heart failure. Without surgery, it is associated with high morbidity and mortality.

Methods: We conducted a retrospective observational study of clinical presentations and perioperative outcomes of pericardiectomy in all patients operated from July 2015 to December 2018 for tuberculous CCP.

Results: A total 14 patients (mean age - 38 ± 13.3 years, 10 male), underwent pericardiectomy via median sternotomy without cardiopulmonary bypass. Eleven patients (79%) had completed treatment for pulmonary tuberculosis, and three (21%) were on antitubercular treatment at the time of referral for surgery. Ten patients (71%) had prior hospitalisation for cardiac failure. At the time of surgery, eight patients (57%) were in New York Heart Association (NYHA) class III-IV. The median duration of symptoms before surgical intervention was 15 months (range 11–24 months). Three patients (21%) had associated cardiac cirrhosis. Twelve patients (86%) underwent total pericardiectomy. Two patients (14%) underwent partial pericardiectomy. The mean operative time was 160 ± 33.8 minutes. The mean central venous press pressure before and after surgery were 28 ± 3.9 and 10 ± 2 mmHg respectively. The mean intensive care unit (ICU) and hospital stays were 4 ± 1.5 and 10 ± 2 days respectively. There was one (7%) 30-day mortality. There were two deaths (14%) due to non-cardiac causes at 10 and 16 months respectively. The remaining 11 patients (79%) are doing well (mean follow-up- 23 months), and are in NYHA class I.

Conclusions: Tuberculosis is the most common cause of CCP in our region. Pericardiectomy provides definitive treatment to alleviate symptoms resolution and improve survival.

* *Corresponding author*. 00977 9812140797 (mobile).
E-mail address: lokesh_shekher@yahoo.com (L.S. Jaiswal).

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1. Introduction

Chronic constrictive pericarditis (CCP) is the end-stage of chronic inflammatory process that produces fibrosed, thickened and constricted pericardium around the heart, which leads to abnormal diastolic ventricular filling.[1] Aetiology of CCP has a regional variation. Idiopathic, post-surgical and prior mediastinal radiotherapy are the common causes in the Western world[2–4] while tuberculous infection remain the important cause in countries where it is a major public health problem.[5–7]

Constrictive pericarditis is one of the most serious sequelae of tuberculous pericarditis. Despite treatment with antitubercular drugs and use of corticosteroids, almost 30–60% of them develop constriction.[6] Medical treatment without surgical intervention leads to progression of symptoms and early death.[8–10] The resection of the diseased pericardium is essential for minimizing morbidity, mortality, improving long-term functional results and quality of life.[11–14]

In this study, we aim to review the clinical profile and outcome of pericardiectomy for tuberculous CCP.

2. Materials and methods

We conducted a retrospective study of all patients undergoing pericardiectomy for tuberculous CCP from June 2015 to June 2018. Our centre is a tertiary care centre providing cardiac surgery services in the Eastern Nepal catering to a population of around 1.5 million.

The diagnosis of CCP was based on clinical features, echocardiographic criteria of constrictions, radiologic (chest radiograph and computed tomography [CT]) evidence of pericardial thickening and calcification. The echocardiographic diagnosis of constrictive pericarditis was based on the combinations of the following criteria-septal bounce, ventricular septal shift with respiration, respiratory variation of mitral E velocity >25%, normal or increased mitral annular e' velocity, augmented hepatic vein diastolic flow reversal of >25% on expiration, dilated inferior venacava with inspiratory collapse <50% and pericardial thickening and calcifications. Computed tomography confirmation of pericardial thickness and calcifications was performed in all cases.

All patients were operated under general anaesthesia with central venous catheter access and invasive pressure monitoring. Median sternotomy without cardiopulmonary bypass (CPB) was the preferred surgical approach. Cardiopulmonary bypass was kept standby for the following scenarios: (1) inadvertent injury to the cardiac chambers; (2) hemodynamic instability; (3) presence of a calcific pericardial "cocoon" encompassing all cardiac chambers; and (4) coexisting cardiac lesions requiring correction. Anterolateral clearance of constriction from phrenic nerve to phrenic nerve, diaphragmatic clearance behind the heart till oblique sinus, followed by clearance over right atrium and inferior venacava was done. Posterolateral clearance posterior to the left phrenic nerve was done as far as possible.

After median sternotomy, the thymus and pleural reflection were mobilized and bilateral pleura were wide open. Wide opening of bilateral pleura allowed easy identification of phrenic nerve and assisted in dissection of lateral wall posterior to left phrenic nerve. The initial dissection was performed over aorta, pulmonary artery and superior venacava, as pericardium over these structures is relatively less adherent and CPB can be initiated whenever required. The pericardium over right ventricle was inspected for a relatively soft and uncalcified area. An initial small cruciate incision was made in the thickened pericardium until the parietal pericardium and the underlying epicardial fat was located. The incision was extended and the thickened pericardium was dissected in an avascular plane with electrocautery. The anterior pericardium was initially dissected in midline from aorta superiorly to diaphragm inferiorly. The dissection was then extended on the left side till left phrenic nerve and on the right side till atrioventricular groove. This was followed by clearance of pericardium over diaphragmatic surface till oblique sinus by retracting the heart superiorly. Clearance of the pericardium posterior to the left phrenic nerve was completed as far as possible. Finally, pericardium over right atrium down to the level of pulmonary veins and inferior venacava was cleared. Calcified spicules or plaques infiltrating the myocardium were left behind as islands to prevent inadvertent myocardial injury.

Patients were followed at 1,3,6 and 12 months and yearly thereafter. Total pericardiectomy was defined as a wide excision of the pericardium over the anterolateral surface from phrenic to phrenic nerve, diaphragmatic surfaces of both ventricles, and over the great vessels including the intrapericardial portion of the vena cava–right atrial junctions. Any excision less than this were considered partial pericardiectomy. The tuberculosis was considered as the aetiology if any of the following features were present: 1. Histological section of the excised pericardium showing granuloma, caseation, giant cells and/or tubercle bacilli, 2. Evidence of tuberculosis elsewhere (previous history or on active anti-tubercular treatment [ATT].

All data collected were processed in accordance with institutional guidelines to ensure patient privacy and confidentiality. Data on patients' demographic, intraoperative and postoperative parameters were collected retrospectively from patients' medical records. Data was analysed with Microsoft Excel (2007). Continuous variables were presented as mean with standard deviation or median with range as appropriate. Categorical variables were presented as percentages and numbers.

3. Results

Fourteen consecutive patients (mean age: 38.2 ± 13.3 years, male: 71%) underwent pericardiectomy for tubercular CCP. Ten patients (71%) had a previous history of hospitalisation for cardiac failure. Eight (57%) were in NYHA class III-IV before surgical intervention. Eleven patients (79%) had a prior history of ATT for pulmonary tuberculosis. The median duration from completion of ATT to pericardiectomy was 34 months (range - 20–46 months). Three patients (21%) were on ATT at the time of referral. None of the patients received corticosteroids or anti-inflammatory agents. All patients completed six months

of ATT. The median duration of symptoms to surgical intervention was 15 months (range 11–24 months). Patient characteristics are tabulated in Table 1.

Chest radiograph revealed cardiomegaly in six patients (43%) (Fig. 1), and pleural effusion in 12 patients (86%). Electrocardiogram showed low voltage QRS complex in five patients (36%). Atrial fibrillation was seen in one patient (7%), which persisted even after pericardiectomy. Five patients (36%) had calcification on chest X-ray. Computed tomography scans of the chest showed calcifications in all patients. Three patients (21%) had a calcific annular constricting ring around the atrioventricular groove (Fig. 2). Three patients (21%) had liver ultrasonograms suggestive of cirrhosis with deranged liver function tests.

Total pericardiectomy was performed in 12 patients (86%). Two patients (14%) underwent partial pericardiectomy due to the dense calcifications. The mean operative time was 160 ± 33.8 minutes. The average blood loss was 200 ± 48.6 mL, five patients (36%) required packed red blood cell transfusions. The mean central venous pressures before and after pericardiectomy were 28 ± 3.9 and 10 ± 2 mm Hg respectively. The mean intensive care unit (ICU) and hospital stays were 4 ± 1.5 and 10 ± 2 days respectively. Intraoperatively, all patients had thickened pericardium with various degrees of calcifications and myocardial adhesions. Histological examination of pericardium showed caseating granuloma with giant cells suggesting of tuberculosis in all patients.

No patients required conversion to cardiopulmonary bypass. Two patients (14.3%) had features of low cardiac output syndrome in the postoperative period which was managed with inotropic support. There was one (7%) perioperative mortality due to ventricular arrhythmia on seventh postoperative day. There were two (14%) late deaths at 10 and 16 months after pericardiectomy, respectively. The first mortality was due to type 2 respiratory failure and sepsis following thoracotomy for bilateral calcific fibrothorax. The second death was due to acute exacerbation of COPD. The remaining 11 patients are doing well at a mean follow-up of 23 months (range: 10–42 months). All the patients are in NYHA class I with no clinical features of cardiac failure. Follow-up echocardiogram at six months showed no evidence of constrictive physiology in all surviving patients.

Table 1 – Demographics and clinical profile.

S.No	Characteristic	Values [mean ± SD or n(%)]
1	Age, years	38.2 ± 13.3
2	Gender	
	Male	10 (71.4%)
	Female	4 (28.6%)
	Median Duration of symptoms	15 months
3	NYHA function class (at admission)	
	Class I-II	6 (42.9)
	Class III-IV	8 (57.1)
4	Rhythm	
	Sinus	13 (92.9%)
	Atrial fibrillation	1 (7.1%)
	Others/VPCs	2 (14.3%)
5	Mean Preoperative CVP (mmHg)	28 ± 3.9
6	LV function	
	>50%	12
	30–50%	2
	<30%	Nil
7	Pleural effusion	12 (85.7%)
8	Associated symptoms	
	Dyspnea	14 (100%)
	Hepatomegaly	12 (85.7%)
	Peripheral edema	14 (100%)
	Ascites	10 (71.4%)
	Chylous ascites	1 (7.1%)
	Cough	6 (42.9%)
	Fever	4 (28.6%)
9	Associated medical conditions	
	Hypertension	3 (21.4%)
	COPD	1 (7.1%)
	Diabetes	2 (14.3%)
	Hypothyroidism	1 (7.1%)
	Liver cirrhosis	3 (21.4%)
	Hyperbilirubinemia	5 (35.7%)
	Hypoalbuminemia	8 (57.1%)
	Renal dysfunction	1 (7.1%)
	Fibrothorax	1 (7.1%)

4. Discussion

The most common aetiology of CCP in developing countries is tuberculosis. It is reported in 61%–87% cases of CCP.[5,10] The disease commonly present in the third and fourth decade of life,[5] as seen in our study. In around half of the patients with tuberculous pericardial effusion, resolution occurs without constriction. The remaining half develops CCP despite ATT.[15] In contrast, CCP develops in only 1.8% of patients with acute pericarditis of non-tuberculous cause.[16] The pericardium in patients with tuberculous pericarditis are markedly thickened, contains areas of calcification and necrosis along with loculated collections. There is a tendency for the tuberculous pericarditis to invade myocardium. The pericardium in patients with nonspecific or idiopathic aetiology shows smooth thickening and less calcification.[17] In our patients, the pericardium was heavily thickened with calcification, and adherent to the myocardium at multiple areas, suggesting tuberculous aetiology. All our patients either had a history of tuberculosis or received ATT before surgery. Furthermore, histology confirmed chronic inflammation with granulomas and giant cells. The median duration of symptoms to surgical intervention was prolonged (15 months), as our patients were extensively investigated and treated for various suspected hepatic, malignant, and cardiac disorders before being referred to our unit.

The diagnosis of constrictive pericarditis is primarily made by echocardiography with supporting evidence of pericardial thickening and calcification by computed tomography and magnetic resonance imaging. Cardiac catheterization is rarely used nowadays. Constrictive pericarditis causes dissociated intrathoracic and intracardiac pressures, and enhanced ventricular interaction originally described by Hatle et al[18] These are the most important diagnostic features which can be assessed by echocardiography.[19] The combination of characteristic haemodynamic changes with pericardial thickness greater than 3 mm is usually confirmatory.

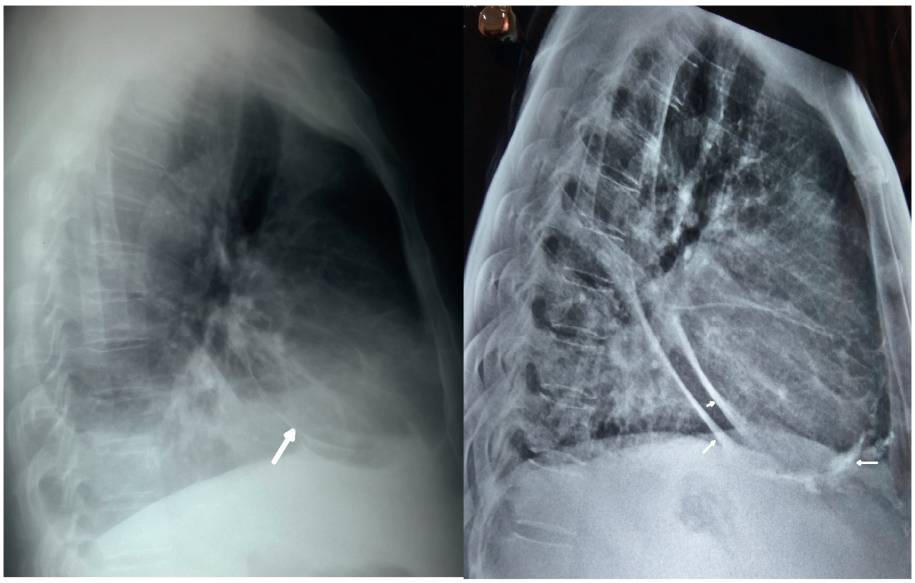

Fig. 1 – Chest radiographs showing calcification (arrows) of pericardium.

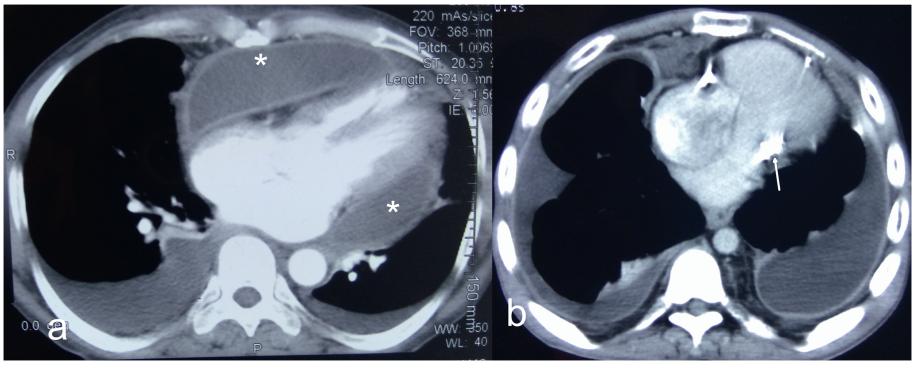

Fig. 2 – CT chest showing (a) thickened pericardium with loculations (asterix) and (b) annular calcific constriction (arrow).

Chronic constrictive pericarditis is a treatable cause of diastolic heart failure. Surgery should be considered in all patients with unexplained right heart failure and preserved left ventricular function. Pericardiectomy is the only definitive treatment option for these patients. Successful surgery leads to resolution of symptoms and improved quality of life. In the present study, pericardiectomy was successful in all patients, with excellent postoperative outcome.

Since Churchill performed the first successful pericardiectomy for constrictive pericarditis, the surgical approach has changed from limited thoracotomy incision with partial pericardial excision above the phrenic nerve to complete resection of entire pericardium and outcomes have improved over time.[4,13,14] However there is no consensus regarding approach and extent of pericardiectomy. Median sternotomy provides good exposure of the right atrium and the venacavae, anterior and diaphragmatic surface, and enables easy clearance of the thickened and densely adherent pericardium. Moreover, the patient can be connected to CPB in case of inadvertent cardiac injury. Area posterior to phrenic nerve is relatively difficult to reach without manipulation of heart compared to anterolateral approach. Left anterolateral thoracotomy is done less commonly, as the right-sided pericardium could not be effectively removed. This approach is preferred in the setting of concomitant empyema thoracis and bacterial pericarditis due to the concern of sternal infection. It is also preferred in the setting of early effusive constrictive pericarditis where adhesions are fibrinous with loculations, which makes total pericardiectomy feasible. In our study, all cases were operated via median sternotomy without the need for CPB.

The extent of pericardiectomy has been variably defined as "radical", "total", "subtotal", "partial" and "adequate" pericardiectomy. The precise definition is however lacking.[22] Nevertheless, pericardial resection should be as extensive as possible to permit complete expansion of ventricles. In our study total pericardiectomy was defined as a wide excision of the pericardium over the anterolateral surface from phrenic to phrenic nerve and diaphragmatic surfaces of both ventricles, and over the great vessels including the intrapericardial portion of the vena cava–right atrial junctions. Any excision less than this was considered a partial pericardiectomy. The extent of pericardial resection, aetiology and associated tricuspid regurgitation has been shown to influence the long

term outcome. Partial or subtotal pericardiectomy and presence of tricuspid regurgitation is associated with inferior long term outcome.[20,21] Therefore total pericardiectomy and repair of associated valvular regurgitation should be completed for good long term outcome. Similarly idiopathic and infective constriction have good long term outcome compared to post-surgical and post-irradiation constriction. In our study all of them were having infective aetiology. Factors predicting poor short term outcome following pericardiectomy are: ascites, low ejection fraction, hyperbilirubinaemia, renal dysfunction, higher preoperative right atrial pressure, atrial fibrillation, tricuspid and mitral regurgitation and postoperative low-output syndrome.[22–24] There was one (7%) in-hospital mortality in the present study. Multiple risk factors (hyperbilirubinaemia, cirrhosis, partial pericardiectomy and renal dysfunction), were present in this patient.

In our series, more than half of the patients were in NYHA class III/IV before surgical intervention. After surgery, all the surviving patients are in NYHA class I at mean follow-up of 23 months.

4.1. *Strengths and limitations*

Data on tuberculous CCP and its outcome are limited. This study provides the clinical profile and outcomes of pericardiectomy in tuberculous CCP in a Nepalese population. The study is limited by its retrospective nature, small number of patients and relatively short duration of follow-up.

5. Conclusions

Chronic constrictive pericarditis is a surgically treatable cause of diastolic heart failure. Tuberculosis is the most common aetiology in an endemic country like Nepal. Pericardiectomy combined with ATT is the best treatment option. Total pericardiectomy should be the goal and this can be effectively achieved via median sternotomy in the majority of patients.

Conflicts of interest

All authors have none to declare.

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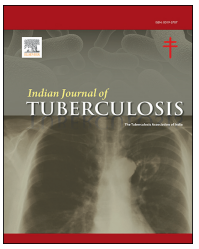

Original Article

Lysosomal acid lipase gene single nucleotide polymorphism and pulmonary tuberculosis susceptibility

Deo Kabuye [a,b,*], *Angelamellisy Ndibalema* [a,c]

[a] *Department of Laboratory Medicine, The First Affiliated Hospital of China Medical University, Shenyang, 110001, Liaoning Province, China*
[b] *Kalisizo Hospital, Uganda*
[c] *Kairuki Hospital, Tanzania*

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ABSTRACT

Background: The factors that predispose to pulmonary tuberculosis (PTB) are not fully understood, However. Gene polymorphisms have been associated with PTB development.
Objectives: In this study, we investigated the relationship between *LIPA* gene polymorphisms and a predisposition to pulmonary tuberculosis caused by *Mycobacterium tuberculosis*.
Methods: A total of 202 cases of PTB and 218 healthy controls (HCS) were included in this study. Analyses were done under allelic, homozygous, and heterozygous, dominant, recessive models, and were used to calculate values, odds ratios (ORs), and 95% confidence intervals (CIs) for assessing the association between single nucleotide polymorphisms (SNPs) and disease risk. Genotyping was conducted using the real time polymerase chain reaction with high resolution melting curve analysis.
Results: When comparing PTB patients with healthy controls (HCS), significant associations with disease development were observed for both SNPs rs1051338 and rs7922269. Analysis was done based on models of genetic inheritance in man that is co-dominant, recessive and dominant models. Rs1051338, the heterozygous (AC vs. AA) P: 0.001, OR: 1.998, 95% CI: 1.312–3.042 and homozygous (CC vs. AA) P: < 0.001, OR: 4.078, 95% CI: 2.134–7.796 Co-dominant associated with increased risk for the disease. Under recessive (CC vs. AA + AC), P: 0.001, OR: 2.829: 95% CI: 1.543–5.185 and dominant model (AC + CC vs. AA) P: < 001, OR: 2.331, 95% CI: 1.564–3.474 the genotypes distribution increased the individual risk, plus its alleles distribution (P: < 0.001, OR: 2.004, 95% CI: 1.505–2.669). Considering SNP rs7922269 mutation significantly increased pulmonary tuberculosis risk as was observed in the homozygous GG vs. TT (P: 0.003, OR: 3.162, 95% CI: 1.431–6.989); heterozygous GT vs. TT (P: < 0.001, OR: 1.2.259, 95% CI: 1.503–3.394); dominant model (GT + GG vs. TT; P: < 0.001, OR: 2.061, 95% CI: 1.402–3.032) and the allele G (P: < 0.001, OR: 1.829, 95% CI:1.361–2.458), however no significant association was observed in the Recessive model (GG vs. TT + GT; P: 0.057, OR: 2.568, 95% CI: 0.965–4.432).

* *Corresponding author.*
E-mail addresses: deoki22@yahoo.co.uk (D. Kabuye), a.revelian@yahoo.com (A. Ndibalema).
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Conclusion: The findings of our study strengthen the hypothesis that LIPA rs1051338 and rs7922269 polymorphism associated with increased risk for pulmonary Tb in a sample of northern Chinese population.

1. Introduction

Mycobacterium tuberculosis, Is among the main cause of morbidity and mortality, and further has remained a major health problem throughout the world. Previous studies indicate that one-third of the global population is infected with *M. tuberculosis* of which 10.4 million people near to 10% develop active disease in their lifetime[1,2] and almost two million deaths occur every year.[3] Globally, 2.0 billion people are estimated to have latent pulmonary tuberculosis but the pathophysiology associated with restriction within this state or development of active disease remains controversial, and Latently infected individual serve as a source of infection in new incidences.[4,5] The remaining 90% of latently infected individuals mount immune response that arrests further the development of tuberculosis disease. Some host, pathogen and environmental factors have been suggested to be responsible for infectivity, reactivation and progression of tuberculosis disease but on the other hand genetic variations have been linked to the increased risk to tuberculosis.[6,7] Following inhalation of infectious nuclei droplets the pathogens are internalized by various phagocytic cells such as Macrophages, neutrophils and dendritic cell which initiate and mobilize collaborated immune response.[8] The survival of ingested tubercle bacilli in the macrophages depends on the failure of phagosome lysosome fusion, and its capability to use intracellular metabolites such as amino acids, cholesterol and fatty acids. Inside the phagocytes *Mycobacteria* use cholesterol and fatty acids as a source of carbon and energy for its survival and proliferation. Furthermore, the intracellular cleavage of cholesterol by bacilli avails propionyl coA which are essential in the synthesis of phthiocerol dimycolate-pDIM and sulpholipid (SL) bacterial virulence factors.[9,10] Cholesterol from host cell is utilized by the bacilli through its mce4 transporter system with the fatty acid from the host cells being transported by ATP binding cassette transporter into the bacilli stored as triglycerol (TAG) in lipid droplets.[9] The individual susceptibility or resistance to *Mtb* is influenced by both host genetic factors, pathogen and environmental components.[11]

Lysosomal acid lipase enzyme also known as cholesterol ester hydrolase enzyme, is encoded by LIPA gene located at long arm of chromosome 10 region 2 band 3 sub-band 3 responsible for synthesis of protein with 399 amino acid.[12] It mediates the breakdown of complex molecules such cholesterol esters and triglycerides in the lysosome compartment, and mutation of this enzyme leads to the accumulation of fats in liver cells and macrophages. LAL is only lipase enzyme with ability to function in an acidic environment. After the cleavage of cholesterol esters and triglycerides, the free fatty acids, free cholesterol and monoglycerides are delivered into the cytosol to be utilized, cholesterol maintains the cell membrane integrity and facilitates the invasion of phagocytic cells by *Mycobacterium*.[13]

LIPA gene polymorphism has been associated to some disease conditions such as atherosclerosis, Wolman disease, coronary artery diseases.[9,12,14] However, its function in relation to pulmonary diseases has not yet been investigated therefore, in this study, we postulated that LIPA gene polymorphism contribute to individual variability in response to pulmonary tuberculosis. Thus to test the potential association of LIPA gene variation, one exonic and one intronic SPNs were analysed in a sample of 420 individuals.

2. Materials and methods

2.1. Study population

In this case control study, a total of 420 individuals were selected of which 202 (Females = 59 Males = 143) were recruited as cases with pulmonary tuberculosis from Tuberculosis Research Institute of Shenyang basing on clinical examination, radiological results, smear examination and culture. 218 (Females = 79, Males = 139) healthy individuals were recruited as controls from First Hospital of China Medical University, which matched cases in the same cohort according to sex, race, age and geographical area. All study controls had no history of tuberculosis. The experimental study protocol was approved by hospital research committee of China medical University. All individuals gave informed consent before their inclusion in this research study.

2.2. Exclusion criteria

All individuals with HIV (immunodeficiency Virus), Diabetes, autoimmune diseases and chronic diseases, and other pulmonary diseases were not included in the study.

2.3. DNA extraction

Whole blood samples were collected in EDTA tubes from all individual participants and genomic DNA was extracted from 200 micro litres of blood using QIAmp DNA micro kit (qiagen, Hilden Germany) as per manufacturer's manual. Before analysis, the extracted DNA was kept at −20 °C before use. The DNA concentration and purity were determined using a spectrophotometer.

2.4. SNP selection and genotyping

The study SNP rs1051338 and rs7922269 were selected from National Center of Biotechnology Information dbSNP database (NCBI dbSNP database) with minor allele frequency (MAF) greater than 0.05 in Chinese population (CHB). The SNPs amplification and genotyping were carried out using Polymerase chain reaction technique and the light Cycler 480 System to perform high resolution melting analysis (Roche Applied Sciences, Penzberg, Germany). Experimental primers with annealing temperature between 55 and 65 °C were designed using primer blast and amplicon size with 100–200 base pairs were maintained. Briefly, in the experimental protocol, The DNA was amplified in a 96 white well plate. A total of 20 μL reaction volume was used, Consisted of Master mix ((Roche Applied Sciences) containing FastStart Taq DNA polymerase, reaction buffer, dNTPs and HRM dye) 10 μL; Magnesium chloride (25 mM; Roche Applied Sciences) 2 μL; Reverse primer 1ul, Forward primer 1 μL, double-distilled purified water 5 μL and template DNA (30 ng/mL) sample 1ul. The cycling parameters for PCR amplification were as follows: 95 °C for 4 minutes, then 45 cycles of 95 °C for 10 s, annealing at 60 °C for 30 seconds and extension at 72 °C for 30 seconds. After the PCR amplification HRM analysis began, the precise warming of PCR DNA amplicons from 50 °C–95 °C was undertaken. As the temperature increased, the double-stranded DNA denatured and melted apart causing the fluorescence to fade away. The fading signals correspond to various nucleotides present. Results were analyzed using Light Cycler Z 480 Gene scanning software (Roche Applied Science). Using Gene Scanning Module, data was analyzed of which three sets of curves: wild homozygous type, mutant homozygous type, and heterozygous type were obtained. Ten PCR products were randomly selected from each of the three genotypes for sequencing identification using an ABI7000 sequence detection system (Applied Biosystems).

2.5. Statistical analysis

The statistical analyses were done using SPSS IBM version 20 (Armonk, NY, United States of America). The SNP genotypes and alleles were counted directly. To evaluate the statistical mean difference between cases and controls Chi square was calculated. The strength of tuberculosis and gene polymorphism association was established using odds ratio (OR) and corresponding 95%confidence interval (CI). The unpaired student t test was used for numerical continuous variables. A P value less than 0.05 was considered to be statistically significant. The controls were within the Hardy–Weinberg equilibrium-HWE (>0.05). The Bonferroni correction (Bonferroni tes/adjustment) was used for multiple comparisons. Bonferroni's correction adjusts the critical level of significance for test by dividing the critical level of significance mainly 0.05 by the number of significance tests performed.[15]

3. Results

We used a case–control population study design to assess whether SNPs in gene LIPA were associated with increased individual risk to develop active pulmonary tuberculosis disease in northern Chinese people. A total of 420 participants were enrolled, comprising of 218 healthy controls (47.25 ± 19.36 years) and 202 cases with pulmonary tuberculosis (48.43 ± 13.37 years). The genotype distribution revealed that the analyzed LIPA/LAL gene SNPs both in the case and healthy control group conformed to the Hardy–Weinberg equilibrium HWE with a P value > 0.05. The minor allele frequency for all SNPs was >0.05 in Chinese Han Beijing. The demographic and characteristics of healthy controls and pulmonary TB patients are indicated in Table 1. There was no significant statistical difference in age and gender in the case group compared to the control group.

3.1. Genotype effect of LAL rs11203042 polymorphisms on pulmonary tuberculosis

We stratified the genotypes for the gene variations based on genetic models of inheritance such as Co-dominant, Recessive, Dominant plus the allele.

Rs1051338, the heterozygous (AC vs. AA) P: 0.001, OR: 1.998, 95% CI: 1.312–3.042 and homozygous (CC vs. AA) P: < 0.001, OR: 4.078, 95% CI: 2.134–7.796 Co-dominant were associated to increase the risk for the disease. Under recessive (CC vs. AA + AC), P: 0.001, OR: 2.829: 95% CI: 1.543–5.185 and dominant model (AC + CC vs. AA) P: < 001, OR: 2.331, 95% CI: 1.564–3.474 the genotypes distribution increased the individual risk, plus its alleles distribution (P: < 0.001, OR: 2.004, 95% CI: 1.505–2.669). Considering SNP rs7922269 mutation significantly increased pulmonary tuberculosis risk as was observed in the homozygous GG vs. TT (P: 0.003, OR: 3.162, 95% CI: 1.431–6.989); heterozygous GT vs. TT (P: < 0.001, OR: 1.2.259, 95% CI: 1.503–3.394); dominant model (GT + GG vs. TT; P: < 0.001, OR: 2.061, 95% CI: 1.402–3.032) and the allele G (P: < 0.001, OR: 1.829, 95% CI:1.361–2.458), however no significant association was observed in the Recessive model (GG vs. TT + GT; P: 0.057, OR: 2.568, 95% CI: 0.965–4.432). Table 2. After benferron's correction, genotypes for rs1051338 and rs7922269 were still susceptible factors for pulmonary tuberculosis except GG vs. TT + GT.

Table 1 – The general demographic characteristics of the study population.

Parameter	Cases, N = 202	Controls, N = 218	P value
Sex; Male/Female	143/59	139/79	0.892
Age years	47.25 ± 19.36	48.43 ± 13.37	0.472
Smoking			
Ever smoked	20	–	
Nonsmokers	127	–	
Smoking	55	–	
Culture and smear			
Smear/culture positive	47	–	
Smear positive	48	–	
Culture positive	107	–	
Radiography results			
Cavity tuberculosis	60	–	
Infiltrative tuberculosis	142	–	

(–) not applicable

Table 2 – The distribution of genotypes and alleles in cases with pulmonary TB and controls.

Gene/refSNP	Allele/Genotype	PTB (n)	HC (n)	OR	P value	95%CI
LAL		202	218			
Rs1051338	Allele A	226	313	Reference		
	Allele C	178	123	2.004	<0.001	1.505–2.669
	Co-dominant					
	AA	63	112	Reference		
	AC	100	89	1.998	0.001	1.312–3.042
	CC	39	17	4.078	<0.001	2.134–7.796
	Recessive					
	AA + AC	163	201	Reference		–
	CC	39	17	2.829	0.001	1.543–5.185
	Dominant					
	AA	63	112	Reference	–	–
	AC + CC	139	106	2.331	<0.0001	1.564–3.474
Rs7922269	Allele T	251	327	Reference		
	Allele G	153	109	1.829	<0.001	1.361–2.458
	Co-dominant					
	TT	79	120	Reference		
	GT	113	91	2.259	<0.001	1.503–3.394
	GG	20	11	3.162	0.003	1.431–6.989
	Recessive					
	TT + GT	182	207	Reference		
	GG	20	11	2.068	0.057	0.965–4.432
	Dominant					
	TT	69	120	Reference		
	GT + GG	133	98	2.360	<0.001	1.591–3.502

P < 0.05/5 = 0.01 was considered to indicate a statistical significance after the Bonferroni multiple testing. 95% CI, 95% confidence interval; OR, odds ratio.

Since lipids concentration may have an effect on immune cell function in response to various diseases, we measured the concentration of total cholesterol, total glycerides, low density and high density lipoprotein in the sample of our study population. Statistical significant difference in the serum levels for (TG) triglycerides (P = 0.002) (TC) total cholesterol (P < 0.001) (LDL) low-density lipoprotein (P < 0.001) and (HDL) high-density lipoprotein (P < 0.001) between case and control groups Table 3. After the benferron's correction the serum lipids were found still to increase the risk for the disease. To explore further we analysed the effect of individual genotypes on serum lipid levels, Our results showed that rs792269GG, genotype had high triglycerides (TG), totalk cholesterol (TC) and high density lipoprotein (HDL) in healthy controls with a statistical significance P < 0.013, after benferron's correction, more so rs792269TT and rs792269GG had higher HDL in the control group compared to case group (P < 0.013), similarly rs 1051338AC genotype influenced HDL distribution with high concentration in healthy controls (P < 0.013) Table 4.

Table 3 – The distribution of biochemical parameters in cases with PTB and health controls.

Parameter	Total mean	Controls	Cases	P value
TG (mmo/L)	1.36 ± 1.32	1.52 ± 1.67	1.13 ± 0.55	0.002
TC (mmo/L)	4.61 ± 1.01	4.79 ± 0.93	4.37 ± 1.05	<0.001
LDL-C (mmo/L)	2.99 ± 0.84	3.14 ± 0.76	2.80 ± 0.91	<0.001
HDL-C (mmo/L)	1.15 ± 0.41	1.30 ± 0.33	0.97 ± 0.44	<0.001

P < 0.05/4 = 0.013, statistical significance after benferron's correction. HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides.

4. Discussion

In recent years, many studies focusing on the LIPA gene function have been carried out and results have enormously indicated the importance of LIPA and its associated genetic variations in some clinical diseases. To the best of our understanding, there are no published data about LIPA gene polymorphism and pulmonary tuberculosis.

Pulmonary tuberculosis has remained a health threat for year's world over, knowing the mechanism under which the host genetic factors contribute to resistance or susceptibility to pulmonary Tb could be a cornerstone in its control. Studies have found that host genetic factors play a fundamental role in susceptibility and progression of tuberculosis.[16,17] Therefore, the combination of environmental and genetic factors may determine the individual predisposition to tuberculosis. LAL (Lysosomal acid lipase) a serine hydrolase enzyme cleaves triglycerides and cholesterol esters delivered to lysosome by endocytosis through low density lipoprotein to produce free fatty acids and cholesterol that is important for cell growth and control of intracellular mechanisms that are dependent on cholesterol molecules.[12,18] This cholesterol from the lysosomal compartment can however, be esterified by acyl CoA cholesterol acyl transferase (ACAT) for cytoplasmic storage as cholesterol esters, lipid droplets or transported to the extracellular environment through ATP binding cassette transporters.[19] LAL/LIPA aided lipolysis avails fatty acid necessary

Table 4 – The effect of specific geneotypes on the serum lipid levels.

	Rs7922269			Rs1051338		
	TT	GT	GG	AA	AC	CC
TG						
Cases	1.17 ± 0.94	1.11 ± 0.46	**1.05 ± 0.54**	1.19 ± 0.50	1.23 ± 0.77	1.08 ± 0.54
Controls	1.42 ± 0.83	1.69 ± 2.44	**1.92 ± 1.11**	1.68 ± 2.19	1.48 ± 0.83	1.27 ± 70
TC						
Cases	4.36 ± 1.07	4.39 ± 1.07	**4.19 ± 0.86**	4.43 ± 1.09	4.56 ± 1.15	4.08 ± 1.02
Controls	4.74 ± 0.90	4.78 ± 1.00	**5.26 ± 0.92**	4.78 ± 1.04	4.78 ± 0.90	4.73 ± 0.9
LDL						
Cases	2.82 ± 0.86	2.86 ± 0.92	2.66 ± 0.45	2.86 ± 1.01	2.88 ± 0.88	2.82 ± 0.87
Controls	3.15 ± 0.75	3.03 ± 0.78	3.49 ± 0.83	3.07 ± 0.76	3.17 ± 0.87	3.07 ± 0.88
HDL						
Cases	**1.01 ± 0.62**[p]	**0.94 ± 0.31**[p]	**0.92 ± 0.23**[p]	1.02 ± 0.61	**0.95 ± 0.37**[p]	1.03 ± 0.28
controls	**1.29 ± 0.33**[p]	**1.29 ± 0.34**[p]	**1.29 ± 0.25**[p]	1.26 ± 0.44	**1.18 ± 0.41**[p]	1.33 ± 0.49

In bold with p means still significant after benferron's correction (0.05/4 = 0.013), P value < 0.013.

for fatty acid oxidation in the $CD8^+$ T cells and supports their programming towards the memory cell status.

LIPA gene structurally possesses 10 exons and 9 introns, located on the long arm of chromosome 10, region 2, band 3, and sub-band 3 (10q23.3). LIPA rs7922269 is found in the intron region. Although introns do not encode functional specific protein directly can influence mRNA processing and expression, protein stability and polyadenylation.[20] Furthermore, Introns have been found to regulate the export of mRNA to cytoplasmic region and transcription initiation[21] thus important for full expression and function of genes. Recent studies have found intron variants as one of risk factors responsible for various diseases.[22] However, rs1051338 is located in the exon a major protein coding region causes amino acid change from threonine to proline this may disrupt enzyme function in macrophages. The polymorphism of LIPA gene has been associated to various diseases and conditions like cholesterol storage disease, coronary artery diseases, atherosclerosis, Wolman disease[23,24] and foam cells formation due to imbalance in the influx of low density lipoprotein together with its catabolism,[25] which further hinders phagocytes during inflammatory conditions to execute expected immune response. The lipid laden macrophages are capable of maintaining the survival and proliferation of the bacilli thus consequently serving as a source of infection. The LIPA gene mutations have been documented to cause loss of function in the human immune macrophages or monocytes.[26] In the infected immune cells, bacilli accumulate lipids during dormancy stage which later acted upon by Mycobacterial enzymes such as serine hydrolase lipases to synthesize fatty acids and cholesterol, fatty acids enter beta oxidation to produce acetyl CoA that enters glycosylate cycle to provide energy for *M. tuberculosis*.[27] In the host, bacilli use lipids to synthesize their own TAG which are critical for mycobacterial survival during dominant and reactive stages.

The foam cells encountered during pulmonary tuberculosis are mainly due to increased accumulation of triacyl glycerides (TAG) and cholesterol. LIPA gene variations may escalate the level of lipid expression which influences the intracellular hydrolysis and release of free cholesterol and free fatty acids from the lysosomes' compartment.[14,28] In this study, we investigated the role of single nucleotide polymorphisms of LIPA gene and pulmonary Tb susceptibility in Chinese population, as a first attempt to associate the lysosomal acid lipase and *M. tuberculosis*.

In this study, we found rs1051338 greatly associated with risk for pulmonary tuberculosis in northern studied Chinese population where higher frequencies of genotypes were observed in the case compared to healthy control group, this in agreement with another study where this genetic variation was found as a risk factor for developing coronary artery diseases.[22,29] This may be due to increased free cholesterol in lysosome that alters PH hence lysosomal dysfunction supporting the survival of the bacillus and further, increased lysosomal pH disrupts lysosomal integrity and degradation capacity and as a consequence impact the ability of macrophages to handle phagocytosed pathogens. Also this defect could inhibit the cholesterol and fatty acid transportation to the cytoplasm or efflux leading to their accumulation in the lysosome. Similarly another snp rs7922269 was found to increase the individual susceptibility to pulmonary tuberculosis, except the recessive model GG vs. TT + GT which had no statistical significant association. This could explain that mutation defect for LIPA gene in immune macrophage accelerates the foam cell formation by increased lipid droplets accumulation as result hinders the cell phagocytic function to eliminate *M. tuberculosis*. Furthermore, in vivo studies have demonstrated that the function of immune cells is greatly impaired in LIPA knockout mice, as this could be due to reduced LAL activity and cholesterol efflux.[30]

Also, this could suggest that polymorphism increases the enzymatic activity in the lysosomal and the rate at which cholesterol and free fatty acids are transported into the cytoplasm to ccelerate cell foam formation. Furthermore, foam cell culminates into loss of macrophage function like decreased respiratory burst and phagocytosis in tuberculosis foam cell. Macrophages in tubercle infections normally act as inducers and effectors of inflammation, adaptive and natural immunity[27] hence during gene variation these functions are affected. In a similar way, Dehghan and colleagues demonstrated genetic variation in the intron site as risk factor for coronary artery disease.[31]

Nutrition status can influence the individual risk to pulmonary tuberculosis, therefore, in this Lipid levels were measured in the fasting serum samples. From the results, low levels were greatly observed in case group and a significant statistical difference was noted for High density lipoprotein, Low density liproprotein, Total cholesterol, and Triglycerides ($p < 0.05$). Cell cholesterol must be maintained within the acceptable limits since variation of cholesterol in a cell results in disruption of cell membranes integrity, apoptosis and necrosis. In our findings rs 7922269GG had high significant TC, TG, HDL, LDL, with high values in controls. This could explain phenomenon that the variant inhibits efflux of lipids from the cell into the extracellular circulation incourse of tubercle infection. Therefore, it seems likely that genetic variation influences the net functional effect thereby increasing lipid accumulation in the macrophages to enable efficient adaptation of *M. tuberculosis* while affecting the strong immune response. Finally our findings need to be generalized cautiously to other population with different genetic make up and enironmental risk factors. In summation, we found that variation in the LIPA gene may contribute to pulmonary tuberculosis risk. Future larger investigation may advance the importance of LIPA gene polymorphism.

5. Conclusion

Our study findings shows that rs7922269 and rs1051338 increase the risk for individual susceptibility to pulmonary tuberculosis. We recommend that in future another study should be under taken with big sample size in different population, culture of cell lines transfected with mutant bases to determine their effect on the enzyme/protein expression.

Author contributions

Study design, Sample collection, DNA isolation, experiments, data analysis and writing the manuscript were done by Deo Kabuye.

Study design, DNA isolation, drafting the manuscript were done by Angelamellisy Ndibalema.

Final review of the manuscript was done by all authors.

Conflicts of interest

All authors have none to declare.

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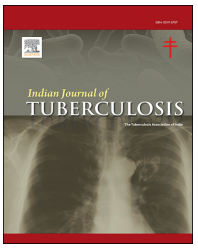

Original Article

Comparison of diagnostic accuracy of digital chest X-ray images between PACS and WhatsApp Messenger in resource poor setting

Sameer G. Kotalwar, Senior Resident [a], *Ashish Kumar Prakash, Consultant* [a,*], *Poulomi Chatterjee, Consultant* [a], *Bornali Datta, Director* [a], *Pinky Goyal, Consultant* [a], *Jaya Prasad Tripathy, Assistant Professor, Department of Community Medicine* [b], *Shreya Singh* [c], *Sandeep kr Mittal, Consultant* [a], *Ankit Aggrawal, DNB fellow* [a], *Anand Jaiswal, Director* [a]

[a] *Department of Respiratory & Sleep Medicine, Medanta the Medicity, Gurgaon, India*
[b] *AIIMS, Nagpur, India*
[c] *KMC, Manipal, India*

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ABSTRACT

Purpose: Many underserved remote locations without specialists would benefit from the ability to quickly and easily share images of radiographs with trained radiologists using WhatsApp messenger. However, there is limited evidence on the role of WhatsApp messenger for sharing chest x-ray (CXR) images to aid diagnosis and management. The objective of the study was to determine the diagnostic accuracy and inter-observer agreement of WhatsApp messenger images of digital CXR compared to viewing on Picture Archiving and Communication System (PACS) monitor.

Methods: Two pulmonologists reported 400 WhatsApp messenger images of digital CXR each. After a wash period of two weeks, they reviewed the original CXR images on PACS and again reported their findings. Diagnostic agreement was measured using kappa value, diagnostic accuracy was evaluated by sensitivity and specificity.

Results: The diagnostic agreement between WhatsApp and PACS images for both the readers was high in case of normal CXR (0.84), Pneumonia (0.85) and Active Koch's (0.79) and Old Koch's (0.71). The inter-observer agreement between two readers on WhatsApp images was good in cases of normal chest x-ray (0.74), Active Koch's (0.61) and Pneumonia

Abbreviations: PACS, Picture Archiving and Communicating System; ICT, Information and Communication Technologies; CT, Computed Tomography; MMS, multimedia messaging; OS, Operating System; iOS, iPhone Operating System; WHO, World Health Organization; GOe, Global Observatory for eHealth; JPEG, Joint Photographic Experts Group; ROC, Receiver operating characteristic; ORIF, open reduction and internal fixation; ICC, interclass correlation coefficients; RUZ, Right Upper Zone; RMZ, Right Mid Zone; RLZ, Right Lower Zone; LUZ, Left Upper Zone; LMZ, Left Mid Zone; LLZ, Left Lower Zone; R1W, Reader 1 on WhatsApp; R2W, Reader 2 on WhatsApp; R1P, Reader 1 on PACS; R2P, Reader 2 on PACS.

* *Corresponding author.*
E-mail address: akp_vpci@yahoo.com (A.K. Prakash).

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(0.74) and low in COPD (0.31) and Pleural Effusion (0.28) and Carcinoma Lung (0.40). In terms of radiological lesion, inter-observer agreement between two readers on WhatsApp images was good in terms of the zonal involvement, moderate in case of infiltrates, consolidation, nodules, and fibrosis, fair in cavity, effusion (0.28) and poor in hilar lymphadenopathy (0.14). The sensitivity in the diagnosis of nodules, effusion and hilar lymphadenopathy was <50% in both the readers.

Conclusion: CXR transmission via WhatsApp is able to identify clinical findings similar to viewing the same image on a PACS monitor in cases of Pneumonia and normal subjects. Active and old Koch's has good comparability whereas; diagnostic agreement is poor in COPD, cavity, pleural effusion and hilar lymphadenopathy, requiring more caution during interpretation.

1. Introduction

The use of mobile devices for capturing medical images has become increasingly popular due to the widespread use of smartphone cameras. The rising popularity of smartphones has driven the technology for transmission of data and the images. It was found that more than 90% of the medical professionals use smart phones.[1]

The widespread use of smartphones and its role in transmitting data and images has important implications on the delivery of specialized health care, especially in remote underserved areas. One such area is the use of digital x-ray in far flung areas. Due to lack of digital x-ray services, people in remote inaccessible areas still depend greatly on the use of film radiographs that too in specialized hospitals located far off. Additionally, there is a scarcity of specialty-trained or formally licensed radiologists to interpret these. Thus, there is a great potential for the use of smartphone capture of radiograph films which would allow increased access to economic and efficient consultation from certified radiologists in such regions.

There have been some recent studies regarding the use of mobile devices for medical image capture and imaging from multiple disciplines such as orthopedics and ophthalmology which have demonstrated an interest in smartphone photography for better clinical decision making.[2–4] Padmasekara et al. exhibited strong inter-rater agreement amongst orthopedic surgeons when comparing iPhone 3GS images of distal radial head fractures interpreted on the iPhone itself via multimedia messaging (MMS) when compared to digitized PACS images.[2] Additionally, Bullard et al. 2013 demonstrated that mobile phone images of CT scans provide adequate imaging for triaging neurosurgical patients to a level 1 trauma center with strong inter-reader agreement.[4] The use of mobile technologies aided by smartphone apps is rapidly gaining ground in health care, also within the field of telemedicine.[5,6]

Despite these evidences, the controversy of losing image quality by digital camera captured images sent via WhatsApp messenger persists. We are still not confident of making crucial decisions regarding patient management by looking at mobile phone images. Noting that the resolution of mobile capture images is most certainly less than the original film radiographs, there are scenarios in which this tradeoff may be acceptable.

Currently, one of the most popular mobile apps is WhatsApp Messenger with more than 1 billion users across 180 countries.[7] WhatsApp Messenger is a communication tool that allows users to send instant messages, photos, video, and voice messages and to make voice calls over an Internet connection.[8] Although scientific studies on the use of WhatsApp Messenger remain scarce in the medical literature (in view of fear of data sharing), increasing numbers of health professionals have adopted it as a communication interface and for the exchange of images and videos. Its use does not seem to reduce image quality in the conversion from analog to digital formats, thus providing the ability to provide sufficient details for better decision making compared to other modalities used for the same purposes.[9–12]

A recent literature review concluded that WhatsApp is a promising tool for communication and sharing information among health care professionals.[5] However, there is limited evidence on the role of WhatsApp as a telemedicine tool for sharing clinical images to aid diagnosis and management. Many underserved or rural remote locations without specialists in place would benefit from the ability to quickly and easily share images of radiographs with trained radiologists across the world using WhatsApp, especially when diagnoses may be vital to altering patient management. This will also offset the issue of paucity of trained radiologists.

Medanta - The Medicity, one of India's largest multi-super specialty corporate hospital located in Gurugram, Haryana launched a "TB-Free Haryana" Campaign in collaboration with the Government of Haryana in 2014–15. Under this public–private partnership, a mobile van equipped with a digital CXR machine was sent to a government health facility in different districts of Haryana on a timetabled visit schedule. The van was used to screen patients with presumptive TB using a "health camp" approach. All presumptiveTB cases (i.e. cough more than two weeks) used to undergo sputum examination and chest x-ray. At the same time, WhatsApp images of x-ray were sent to senior pulmonologists for interpretation, digitized PACS images were sent later.

Against this background, we conducted this study to i) assess the inter-rater agreement between the diagnosis made by two pulmonologists by interpreting digital chest radiography images sent via WhatsApp messenger, ii) assess the agreement between the diagnosis made by interpreting WhatsApp images and digitized PACS images separately for two pulmonologists and ii) calculate sensitivity, specificity of interpretation of WhatsApp images of digital chest x-ray image compared to diagnosis by interpretation of conventional PACS images by two pulmonologists in consensus (gold standard).

2. Methods

2.1. Study design

An observational cross-sectional study (diagnostic agreement between images viewed on WhatsApp messenger vs PACS).

2.2. Study setting

2.2.1. General setting

The project was carried out in the North Indian state of Haryana. The state has a population of 27.2 million residing in 22 districts, predominantly rural (70%), with very low HIV rates, and high levels of poverty.[13] The notified incidence of TB was 145/100,000 population in the state in 2017.[14]

2.3. Specific setting

The TB-Free Haryana campaign was launched in 2015. Under this campaign, a mobile unit consisting of a mobile van equipped with a digital CXR machine was sent to a government health facility in rural remote areas every week. This van was staffed with an x-ray technician, nurse and a driver. A week prior to each screening activity, active information education communication (IEC) campaigns were carried out for public mobilization. The revised RNTCP diagnostic algorithm employing upfront sputum microscopy and CXR followed by GeneXpert (if indicated) was used to screen for active TB.

During the screening session, all presumptive TB cases aged 15 years or above were interviewed for socio-demographic details and the known risk factors for TB using a structured questionnaire. Then they underwent CXR examination and sputum microscopy on a spot sputum sample. Patients were recalled the following morning for a second sputum sample. In the PHIs which also functioned as DMCs, the sputum examination was immediate and results were available on the same day. Where the PHI was not a DMC, sputum spot sample was collected and delivered by Non-Governmental Organizations (NGOs) providing this specific service, the results of which were available in two days.

2.4. Study population

All patients who came for screening to the 'TB free Haryana'mobile van camps with symptoms of cough for more than 2 weeks and/or fever and/or weight loss and/or decreased appetite and/or chest pain and/or hemoptysis. Data collection for the study was done during January–June 2018.

2.5. Data collection procedure (Fig. 1)

Under the TB free Haryana campaign there was a mobile van equipped with digital chest X-ray facility that provided access to digital CXR facilities in far-off locations.

- Patients with cough more than two weeks and/or fever/weight loss/loss of appetite underwent digital CXR and sputum microscopy simultaneously.
- CXR was displayed on a PACS monitor, the image of which was captured using a smartphone (iPhone 5S with 8 megapixel camera) from a distance of 20 cm.
- The photo was cropped to hide the patient details. Another unique number was assigned to each image taken. The number consisted of a code for the pulmonologist R1/R2, W stands for WhatsApp and serial number ranging from 1 to 400 (R1W 1–400 and R2W 1–400), for ex: R1W052.
- The CXR images were subsequently downloaded by a cable to the hospital's PACS system with specific PACS number to each X-ray (R1P 1–400 and R2P 1–400) with P standing for PACS.
- The images captured by the smartphone were sent by WhatsApp messenger to two pulmonologists of the Respiratory Department of our institute which is a private hospital and a tertiary care center (both the pulmonologists had at least 5 years of experience as a chest physician).
- Two minutes were given per pulmonologist per CXR. Images could be zoomed as per need (20 CXRs were reported by each pulmonologist daily).
- CXR reporting was done and entered in the excel sheet with specific WhatsApp number ranging from R1W 1–200 (site-X) and R2W 201–400 (site-Y). Later swapping was done; images of site-X were given to R2 and images of site-Y was given to R1. Thus, a total of 400 WhatsApp images were read by each of the pulmonologists blinded by the interpretation of the other radiologist.
- A washout period of 2 weeks was given between WhatsApp CXR reporting and PACS image CXR reporting.
- For the PACS images also, the pulmonologist read 400 images each (R1P 1–400 and R2P 1–400).
- If there was consensus in the interpretation of PACS images between the two pulmonologists, it was considered as the gold standard.
- Thus, diagnosis was made by Reader-1 on WhatsApp (R1W) and on PACS (R1P), and by Reader-2 on WhatsApp (R2W) and on PACS (R2P) and later swapped as shown in Fig. 1.

While reporting the CXR, the following variables were also captured: zonal involvement (Right Upper Zone, Right Middle Zone, Right Lower Zone, Left Upper Zone, Left Middle Zone, Left Lower Zone), Infiltrates, Consolidation, Cavity, Nodules, Miliary pattern, Fibrosis, Effusion, Hilar lymph node involvement, Normal x-ray finding, Remarks and Final Diagnosis). This system of reporting was followed for both the WhatsApp as well as PACS CXR images.

The strength of agreement (kappa value) was categorized and interpreted as follows:

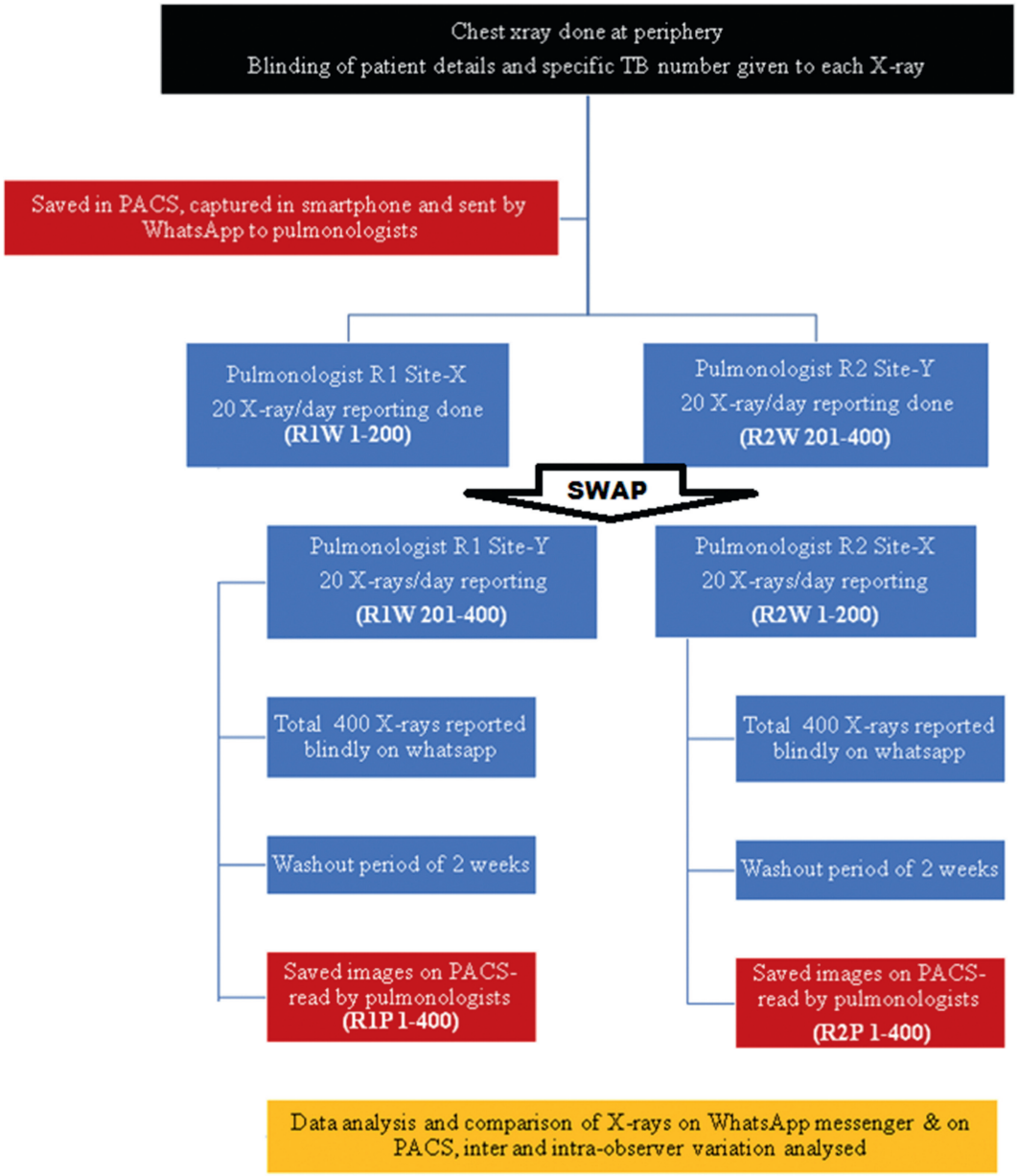

Fig. 1 – Study design.

2.6. *Sample size calculation*

Parameter of interest is the agreement between PACS image and Smartphone capture image sent via WhatsApp messenger while interpreting digital CXR.

Kappa value	Strength of agreement
0.0–0.20	Poor
0.21–0.40	Fair
0.41–0.60	Moderate
0.61–0.80	Good
0.81–0.99	Very Good
1.0	Perfect

Taking k0 = 0.5 (value of kappa under the null hypothesis) and k1 = 0.7 (true kappa statistic), power specified at 90% and alpha value = 0.05, the sample size was estimated to be 367.(Ref).

2.7. *Data analysis*

Data were double entered from the paper-based form into Microsoft Excel and later imported into STATA (version 13, StataCorp LP, Texas, US) for analysis. The kappa coefficient (with 95% confidence intervals [CI]) of agreement and its interpretation was used to determine the inter-rater agreement between two readers on the interpretation of WhatsApp and PACS images. A two-by-two table was constructed to

Table 1 – Diagnosis made by the interpretation of WhatsApp and PACS images by two readers.

Diagnosis	WhatsApp images		PACS images	
	Reader 1 N = 400	Reader 2 N = 400	Reader 1 N = 400	Reader 2 N = 400
	n (%)	n (%)	n (%)	
Normal	209 (52)	253 (63)	198 (50)	213 (53)
Active Koch's	51 (13)	64 (16)	58 (15)	66 (17)
Old Koch's	44 (11)	28 (7)	42 (11)	33 (8)
COPD	32 (8)	16 (4)	34 (8)	43 (11)
Old Koch's With COPD	16 (4)	7 (2)	17 (4)	10 (2)
Heart Disease	11 (2)	7 (2)	4 (1)	7 (2)
Pneumonia	18 (5)	8 (2)	22 (5)	6 (2)
Hilar Lymphadenopathy	8 (2)	2 (0.5)	7 (2)	2 (0)
Pleural Effusion	4 (1)	4 (1)	3 (1)	3 (1)
Bronchiectasis	1 (0.3)	2 (0.5)	6 (1)	2 (0)
Lung carcinoma	4 (1)	5 (1)	3 (1)	2 (0)
Pleural thickening	1 (0.3)	0 (0)	4 (1)	3 (1)
Active Koch's With COPD	1 (0.3)	4 (1)	2 (0)	10 (3)

PACS: Picture Archiving and Communication System; COPD: Chronic Obstructive Pulmonary Disease.

compare the interpretation of the WhatsApp and PACS images, and this was used to calculate sensitivity, specificity and predictive values.

2.8. *Ethics approval*

Ethical clearance was obtained from the Institute's Ethics Committee of Medanta Hospital and detailed discussion was done regarding the breach of confidentiality and it was approved as pilot project. Written informed consent was obtained from all patients prior to their recruitment into the study.

3. Results

A total of 400 subjects were enrolled whose x-rays images were interpreted. The mean age of the subjects in the study was 48.5 ± 16 (SD) years. More than one-third (142, 36%) were in the 60–69 year age group, followed by 50–59 years (69, 17%) and 30–39 years (55, 14%); half of them were males (207, 52%). Interpretation of PACS images showed that there was consensus in the interpretation between both the pulmonologists in 347 (87%) out of 400 cases, thus, were regarded as gold standard for calculation of sensitivity and specificity of WhatsApp images.

According to Reader-1, 52% (n = 209) of the X-rays were normal while 63% (n = 253) of X-rays were reported normal by Reader-2 on interpretation of WhatsApp images. PACS image interpretation showed that 50% (n = 198) were reported normal by the Reader 1 whereas 53% (n = 213) were normal as per Reader 2. Of the abnormal x-rays interpreted by the readers on WhatsApp, active Koch's was the most common followed by Old Koch's. This was similar to those diagnosed by interpretation of PACS images except for Reader 2 on PACS images who reported COPD as the second most common diagnosis which was followed by Old Koch's Reader 2 also had a higher proportion of Active Koch's and Active Koch's with COPD than Reader 1 on both WhatsApp and PACS images. Table 1.

Agreement between the diagnosis made by interpretation of WhatsApp and PACS images has been presented separately for both the readers in Table 2. The results show that diagnostic agreement was VERY GOOD in case of normal chest x-ray finding and Pneumonia and POOR in cases of Pleural thickening and Active Koch's with COPD in both the readers. In active Koch's, agreement was GOOD (0.71) and VERY GOOD (0.87) for reader 1 and 2 respectively. There was notable differences in agreement between both the readers in the diagnosis of Old Koch's, COPD, Old Koch's with COPD, Heart

Table 2 – Agreement between diagnoses made by interpretation of PACS versus WhatsApp images for two different readers.

Diagnosis	Kappa value (Overall)	Interpretation	Kappa value (Reader 1)	Interpretation	Kappa value (Reader 2)	Interpretation
Normal	0.84	VERY GOOD	0.86	VERY GOOD	0.83	VERY GOOD
Active Koch's	0.79	GOOD	0.71	GOOD	0.87	VERY GOOD
Old Koch's	0.71	GOOD	0.58	MODERATE	0.87	VERY GOOD
COPD	0.55	MODERATE	0.72	GOOD	0.32	FAIR
Old Koch's with COPD	0.66	GOOD	0.51	MODERATE	0.90	VERY GOOD
Heart disease	0.72	GOOD	0.56	MODERATE	1.0	PERFECT
Pneumonia	0.85	VERY GOOD	0.85	VERY GOOD	0.85	VERY GOOD
Hilar lymphadenopathy	0.61	GOOD	0.44	MODERATE	1.0	PERFECT
Pleural effusion	0.54	MODERATE	0.39	FAIR	0.66	GOOD
Bronchiectasis	0.85	VERY GOOD	0.66	GOOD	1.0	PERFECT
Carcinoma lung	0.57	MODERATE	1.0	PERFECT	0.39	FAIR
Pleural thickening	0.00	POOR	0.0	POOR	0.0	POOR
Active Koch's with COPD	0.00	POOR	0.0	POOR	0.0	POOR

PACS: Picture Archiving and Communication System; COPD: Chronic Obstructive Pulmonary Disease.

Table 3 – Inter-observer agreement (Reader 1 vs Reader 2) on interpretation of WhatsApp images of chest x-rays conducted under TB-Free Haryana campaign, Haryana, India, 2017.

Diagnosis	Kappa value	Strength of agreement	p-value
Normal	0.74	GOOD	<0.0001
Active Koch's	0.61	GOOD	<0.0001
Old Koch's	0.56	MODERATE	<0.0001
COPD	0.31	FAIR	<0.0001
Old Koch's with COPD	0.55	MODERATE	<0.0001
Heart Disease	0.56	MODERATE	<0.0001
Pneumonia	0.74	GOOD	<0.0001
Hilar lymphadenopathy	0.44	MODERATE	<0.0001
Pleural Effusion	0.28	FAIR	<0.0001
Bronchiectasis	0.67	GOOD	<0.0001
Carcinoma lung	0.40	FAIR	<0.0001

COPD: Chronic Obstructive Pulmonary Disease.

Disease, Hilar lymphadenopathy, Pleural effusion, Bronchiectasis and Carcinoma lung.

The inter-observer agreement between two readers on WhatsApp images was 'GOOD' in cases of normal chest x-ray, Active Koch's and Pneumonia. Agreement was 'MODERATE' for Old Koch's, Old Koch's with COPD, Heart Disease and Hilar lymphadenopathy. Agreement was 'FAIR' in COPD, Pleural Effusion and Carcinoma Lung. Table 3.

Agreement for features of radiological lesions between PACS and WhatsApp images separately for both the readers has been presented in Table 4. It shows that the agreement was GOOD to VERY GOOD in case of infiltrates, fibrosis and zonal involvement except for Left Lower Zone with MODERATE (0.55) agreement for Reader 1. MODERATE agreement was reported by both the readers in consolidation, nodules and effusion. There was notable difference in agreement between both the readers in case of hilar lymphadenopathy.

The inter-observer agreement between two readers on WhatsApp images was 'GOOD' in terms of the zonal involvement. Agreement was 'MODERATE' in case of infiltrates, consolidation, nodules, and fibrosis. Agreement was 'FAIR' in cavity and effusion and 'POOR' in case of hilar lymphadenopathy. Table 5.

The sensitivity in the diagnosis by Reader 1 compared to the gold standard ranged from 72.8 to 92.7% with the lowest in Old Koch's followed by COPD (75%), whereas in Reader 2, the sensitivity ranged from 25% in the diagnosis of COPD to 97.2% in case of normal x-ray finding. Table 6.

The sensitivity in the diagnosis of nodules, effusion and hilar lymphadenopathy was less than or around 50% in both the readers. There was considerable difference in the sensitivity of the diagnosis of left lower zone abnormality, consolidation and cavity between both the readers. Table 7.

4. Discussion

The current study hypothesized that image capture of PACS and online transmission of mobile images (via WhatsApp Messenger) can be reliably interpreted without loss of diagnostic accuracy by same readers. This would help fasten the care of patients. This is the first such study to assess the inter-observer agreement (two readers) between the diagnosis made by interpretation of WhatsApp images of digital CXR films and the agreement between WhatsApp and PACS images in a community-based setting..

The study had some interesting findings. First, the diagnostic agreement between WhatsApp and PACS images for both the readers and the inter-observer agreement on WhatsApp images was GOOD (0.61–0.80) to VERY GOOD (0.81–0.99) in cases of normal CXR, Pneumonia and Active Koch's.

Although overall diagnostic accuracy varies with the diagnosis, the ability to identify "normal" CXRs versus abnormal findings on WhatsApp images is good, which is often the most important immediate clinical need in remote locations. This speeds up assessment, clinical decision making and time to initiation of treatment.

Table 4 – Agreement between diagnoses made by interpretation of PACS versus WhatsApp images for two different readers.

Radiological lesion	Kappa value (overall)	Interpretation	Kappa value (Reader 1)	Interpretation	Kappa value (Reader 2)	Interpretation
Right Upper Zone	0.85	VERY GOOD	0.89	VERY GOOD	0.81	VERY GOOD
Right Middle Zone	0.80	VERY GOOD	0.76	GOOD	0.84	VERY GOOD
Right Lower Zone	0.65	GOOD	0.68	GOOD	0.62	GOOD
Left Upper Zone	0.82	VERY GOOD	0.85	VERY GOOD	0.80	VERY GOOD
Left Middle Zone	0.73	GOOD	0.68	GOOD	0.78	GOOD
Left Lower Zone	0.61	GOOD	0.55	MODERATE	0.67	GOOD
Infiltrates	0.76	GOOD	0.70	GOOD	0.81	VERY GOOD
Consolidation	0.43	MODERATE	0.40	MODERATE	0.48	MODERATE
Cavity	0.56	MODERATE	0.53	MODERATE	0.61	GOOD
Nodules	0.49	MODERATE	0.45	MODERATE	0.53	MODERATE
Fibrosis	0.68	GOOD	0.62	GOOD	0.75	GOOD
Effusion	0.52	MODERATE	0.50	MODERATE	0.54	MODERATE
Hilar lymphadenopathy	0.28	FAIR	0.19	POOR	0.48	MODERATE

PACS: Picture Archiving and Communication System.

Table 5 – Inter-observer agreement (Reader 1 vs Reader 2) on interpretation of WhatsApp images of chest x-rays conducted under TB-Free Haryana campaign, Haryana, India, 2017.

Radiological lesion	Kappa value	Interpretation
Right Upper Zone	0.76	GOOD
Right Middle Zone	0.70	GOOD
Right Lower Zone	0.70	GOOD
Left Upper Zone	0.78	GOOD
Left Middle Zone	0.70	GOOD
Left Lower Zone	0.68	GOOD
Infiltrates	0.60	MODERATE
Consolidation	0.46	MODERATE
Cavity	0.31	FAIR
Nodules	0.48	MODERATE
Fibrosis	0.57	MODERATE
Effusion	0.28	FAIR
Hilar lymphadenopathy	0.14	POOR

CXR reports compared to a spirometric diagnosis of COPD and highlighted the potential to cause misdiagnosis of COPD, consistent with the results of our study.[17]

Poor inter-observer agreement between two readers on WhatsApp images and low sensitivity in the diagnosis of cavity, nodules, effusion and hilar lymphadenopathy points to the fact that we need to exercise more caution in interpreting these radiological lesions on a WhatsApp image.

There were three key limitations in this study. First limitation stems from the fact that the diagnosis made by the pulmonologist was based on digital images and patient's history and not actually examining the patient which could have been the ideal situation, although this was the case with interpretation of WhatsApp images as well. Second, the digital images were obtained from chest radiographs, so one cannot generalize the result of this study to all types of radiographs, thereby requiring future studies. Third, mobile images depend on the specification of the smartphone and the camera which can affect image quality which in turn can impact the diagnosis.

Table 6 – Sensitivity and specificity of diagnosis made by interpretation of WhatsApp images by Reader 1 and 2 compared to the gold standard (consensus diagnosis made by both the readers on PACS images).

Diagnosis	Gold Standard (PACS)	Reader 1 (WhatsApp)	Sensitivity	Specificity	Reader 2 (WhatsApp)	Sensitivity	Specificity
Normal	179	177	92.7	93.4	204	97.2	98.9
Active Koch's	41	40	73.2	96.8	40	87.8	98.7
Old Koch's	18	25	72.8	96.5	16	83.3	99.7
COPD	20	21	75.0	98.2	9	25.0	98.8

PACS: Picture Archiving and Communication System; COPD: Chronic Obstructive Pulmonary Disease.

High diagnostic agreement in case of Pneumonia and Active Koch's has been demonstrated in other similar studies as well.[15,16] This means that in case of the abovementioned conditions users can be more assured that WhatsApp images are giving them just as good an answer to a clinical question as if he/she were looking at the images directly on a PACS viewer.

The study reported poor inter-observer agreement on WhatsApp images in COPD and Pleural Effusion and low sensitivity in the diagnosis of COPD. Previous study by Pudney et al. also reported low sensitivity (35%) in COPD diagnosis on

There are two key issues related to transmission of clinical images over WhatsApp which needs to highlighted. First, during image transmission, WhatsApp® compresses files to conserve the amount of data that needs to be sent which has its effect on image resolution. This can be seen during magnification of the images. Second, the ethics and legality of sending patient data through WhatsApp is not clear. The principal concern is the unsecured way in which information

Table 7 – Sensitivity and specificity of diagnosis made by interpretation of WhatsApp images by Reader 1 and 2 compared to the gold standard (consensus diagnosis made by both the readers on PACS images).

Radiological lesion	Gold standard (PACS)	Reader 1 (WhatsApp)	Sensitivity	Specificity	Reader 2 (WhatsApp)	Sensitivity	Specificity
Right Upper Zone	70	70	91.4	97.9	64	81.4	97.5
Right Middle Zone	71	83	88.7	92.9	73	83.7	96.4
Right Lower Zone	37	33	67.6	97.5	38	67.6	96.0
Left Upper Zone	63	58	84.1	98.3	50	74.6	98.9
Left Middle Zone	62	65	75.8	93.9	53	75.8	98.0
Left Lower Zone	27	24	55.6	97.2	30	74.1	96.9
Infiltrates	88	87	77.3	92.7	68	76.1	99.6
Consolidation	10	27	80.0	94.5	14	60.0	97.7
Cavity	7	15	85.7	97.5	9	71.4	98.9
Nodules	41	19	36.6	98.7	28	48.8	97.3
Fibrosis	28	33	71.4	95.8	29	78.6	97.8
Effusion	17	17	52.9	97.7	15	52.9	98.3
Hilar lymphadenopathy	10	34	50.0	91.2	10	50.0	98.5

PACS: Picture Archiving and Communication System.

is stored and transmitted with servers susceptible to interception. WhatsApp recently added encryption to data transmitted over its network, but in reality the photos exist unencrypted with little protection at the end of the user's smartphone. In order to transmit images via an app, we need to have secure encryption both over the network, as well as on both end users' smartphones. These points need to be considered by practitioners before using any service for transmitting patient data as they run the risk of exposing themselves to clinical and legal risks. Mobile health applications will continue to be an increasing part of a clinician's toolkit. However, before using it in routine clinical practice any potential app needs evaluation and monitoring by regulatory bodies.

Box 1
Operational definitions for the following seven radiological diagnoses (38–45)

Active Koch's-soft non homogenous opacities or infiltrates with cavities, with or without enlargement of hilar or mediastinal lymph nodes with predominance in upper zones. Small fibro nodular lesions (miliary) resembling scattered millet seeds with no predominant zonal distributions.

Old Koch's-fibrosis or scarring of lung parenchyma with mediastinal or tracheal shift/calcified lesions with upper zone predominance.

COPD- Increased radiolucency of lungs, flat diaphragm, pruning of peripheral vasculature, widening of intercostal spaces and long narrow cardiac silhouette with relative oligemia of the pulmonary vasculature. Bullae defined as radiolucent areas >1cm in diameters and surrounded by arcuate hairline shadows.

Heart disease-presence of bilateral perihilar alveolar edema giving rise to "Butterfly appearance" with cardiomegaly and extensive bilateral interstitial markings, pleural effusions may be accompanied in acute decompensations.

Pneumonia-opacities confined tolobar distribution with air bronchogram in them, generally without any mediastinal shift.

Pleural effusion-homogenous smooth opacity not following the lobar distribution, obliterating or blunting the costophrenic angle, may be associated with mediastinal shift to opposite side with Ellis S shaped curve (meniscus sign).

Pleural thickening- Smooth opacity obliterating costophrenic angle associated with rib crowding and mediastinal shift to same side; may be associated with calcification.

5. Conclusion

CXR transmission via WhatsApp is able to identify clinical findings similar to viewing the same image on a PACS monitor in cases of Pneumonia and normal subjects. Active and old Koch's has good comparability whereas; diagnostic agreement is poor in COPD, cavity, pleural effusion and hilar lymphadenopathy, requiring more caution during interpretation.

Conflicts of interest

All authors have none to declare.

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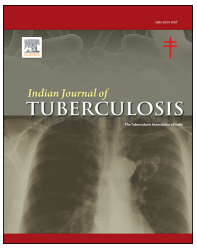

Original Article

Evaluation of magnesium oxide and zinc oxide nanoparticles against multi-drug-resistance *Mycobacterium tuberculosis*

Tofigh Yaghubi kalurazi [a], *Alireza Jafari* [b,c,*]

[a] *Department of Health, Nutrition & Infectious Diseases, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran*

[b] *Inflammatory Lung Disease Research Center, Department of Internal Medicine, Razi Hospital, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran*

[c] *Urology Research Center, Razi Hospital, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran*

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ABSTRACT

Objective: The current study has evaluated the MICs and MBCs of ZnONPs, MgONPs, and MgONPs-ZnONPs against H_{37}Rv Mtb and MDR-Mtb.

Methods: Mixture, magnesium oxide nanoparticles (NPs) and zinc oxide (MgONPs-ZnONPs) were prepared. The microplate alamar blue (MABA) assay and the proportion method were used to evaluate of anti-tubercular activity against MDR-MTB. MTT test was done to MgONPs-ZnONPs against Vero and $HepG_2$ cell lines.

Results: The MIC of MgONPs and ZnONPs were 0.195 and 0.468 μg mL^{-1} against 10^4 of H_{37}Rv Mtb. As well, 0.166 μg mL^{-1} of MgONPs-ZnONPs was able to inhibit 10^{-4} H_{37}Rv Mtb. The MIC of MgONPs against 10^4 concentrations of MDR-Mtb was 12.5 μg mL^{-1}. The MIC of MgONPs/ZnONPs against 10^4 concentrations of MDR-Mtb reached to 0.664 μg mL^{-1}. The MBC value of ZnONPs increased to 1.875 μg mL^{-1} against 10^{-4} concentrations of MDR-Mtb. Testing showed that the MBCs of MgONPs/ZnONPs reached to 1.328 μg mL^{-1} against 10^4 concentrations of MDR-Mtb. The IC_{50} against MDR-TB was 0.779 μg mL^{-1} for ZnONPs and 0.883 μg mL^{-1} for MgONPs-ZnONPs. The MgONPs-ZnONPs was not toxic to Vero cell lines however ZnONPs could inhibit the Vero and $HepG_2$ cell lines.

Conclusion: We found that ZnONPs and mixture MgONPs-ZnONPs not only have higher bactericide behavior but might have also synergistic effects against MDR-TB.

Abbreviations: Mtb, *Mycobacterium tuberculosis*; MDR-TB, Multidrug-resistant *Mycobacterium tuberculosis*; ZnONPs, Zinc oxide nanoparticles; MgONPs, Magnesium oxide nanoparticles; IC50, The half maximal inhibitory concentration; $HepG_2$, Human liver cancer cell line; Vero, African green monkey cell line; MICs, Minimum inhibition concentration; LJ, Löwenstein-Jensen agar; CFU, Colony-forming unit; AFM, Atomic force microscopy; IBB, Institute of Biochemistry and Biophysics.

* *Corresponding author*. Tel: +98133354246, +0098 9123462738 (mobile), Fax: +981333542460
E-mail addresses: Dr.alireza.jafariii@gmail.com, alireza_jafari@gums.ac.ir (A. Jafari).

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1. Introduction

Multi-drug-resistant tuberculosis (MDR-TB) is a form of *tuberculosis* (TB) infection caused by *Mycobacterium tuberculosis* (*Mtb*) that are resistant to treatment with at least isoniazid (INH) and rifampin (RMP). It is estimated that *MDR-TB* caused 480,000 new TB cases and 250,000 deaths in 2015.[1]

Today, nanotechnology has attracted medical researchers for over a century and is now with heavy steps used in biomedical sciences.[2] The researchers have found, metallic NPs have shown anti-tubercular properties against Mtb.[3] In fact, there is a focus of interest related to using mixed metallic NPs because of their outstanding potential against MDR-TB.[2,4,5]

Studies have shown that in the process pathogenesis of pulmonary tuberculosis, the metallic NPs can penetrate within the calcified granuloma and can attach to Mtb and kill it. The metallic NPs can also eliminate Mtb into the macrophage without any toxicity effect. Therefore, the metallic NPs especially mixed metallic NPs have potentially capable of electrostatic interactions with the Mtb cell membrane.[6]

The anti-tubercular potency of metallic NPs such as MgONPs and ZnONPs are still unknown against MDR-Mtb. It is necessary to establish consistent conditions in order to directly compare the effects of the MgONPs and ZnONPs dosages on a clinical strain of MDR-Mtb. Under the consistent conditions, we investigated and compared the effects of different dosage of mixture MgONPs and ZnONPs on two different types of clinical isolates MDR-Mtb and their standard strain. According to the search, this is the first study to use the "Microplate Alamar Blue" method as a rapid and a safety test in order to quantify and compare anti-tubercular activities of mixture MgONPs and ZnONPs against two drug-resistant strains of Mtb clinical isolates with standard strain. Moreover, this is the first time to study the MgONPs-ZnONPs toxicity on African green monkey (Vero) and human liver cancer ($HepG_2$) cell lines.

2. Materials and methods

2.1. Study design and bacterial isolates

The multidrug resistance strain of Mtb isolated from a 55-year-old man who was proven tuberculosis by imaging, clinical findings, histological and cytological observations, previously. The patient had consumed first-line anti-tubercular treatment for 6 months but the symptoms of TB had reappeared about 7 months after completing the treatment. Spectrum sample of patients was cultured in LJ medium for 28 days. The susceptibility drug testing was done by the indirect proportion method to determining of MDR-Mtb.[7]

2.2. Preparing nanoparticles

In this study, MgONPs (average diameter of 20 nm) and ZnONPs (average diameter of 4 nm) were purchased from Tehran University of Medical Sciences (Tehran, Iran). Before each experiment, MgONPs and ZnONPs were sterilized through heating in an oven at 200 °C for one hour.

2.3. Antibiotic susceptibility test

To determine the minimum inhibition concentration (MICs) of NPs, the microplate Alamar blue (MABA) assay is used.[8] Two hundred microliter (200 μL) of sterile deionized water added to outer-perimeter wells of 96-well microplates. Then, 100 mL of 7H9 GC broth was added to each well. 100 μL of MgONPs (12.5 μg mL^{-1}), ZnONPs (30 μg mL^{-1}) and MgONPs-ZnONPs (42.5 μg mL^{-1}) solutions were added to each well, and the serial dilutions were done. A hundred microliter (100 μL) of H37Rv Mtb (Razi serum and vaccine research institute, IRAN) was added to the wells.[9] The plates incubated at 37 °C for 5 days. Fifty microliters (50 μl) of a freshly prepared 1:1 mixture of Alamar blue reagent and 10% Tween 80 added to well and reinsulated at 37 °C for 24 h. The blue color in the well interpreted as no growth and a pink color scored as growth. The

Table 1 – The MICs, MBCs (A) and MTT assay (B) results of MgONPs, ZnONPs and mixture MgONPs-ZnONPs.

(A)

Nanoparticle	Highest concentration (μg.mL^{-1})	$H_{37}Rv$				*MDR-TB*			
		MIC (μg.mL^{-1})		MBC (μg.mL^{-1})		MIC (μg.mL^{-1})		MBC (μg.mL^{-1})	
		10^{-2}	10^{-4}	10^{-2}	10^{-4}	10^{-2}	10^{-4}	10^{-2}	10^{-4}
MgO_2	12.5	0.390	0.195	3.125	1.875	12.5	12.5	≥12.5	≥12.5
ZnO	30	0.937	0.468	7.5	0.937	1.875	0.937	7.5	1.875
MgONPs/ZnO NPs	42.5	0.332	0.166	2.656	1.328	1.328	0.664	5.312	1.328

(B)

Nanoparticle	Initial concentration (μg.mL^{-1})	Stock (μg.mL^{-1})	IC_{50} of $HepG_2$ cell lines (μg.mL^{-1})	IC_{50} of *Vero* cell lines (μg.mL^{-1})
MgO_2	25	12.5	0.279	1.134
ZnO	60	30	3.579	3.579
MgO NPs/ZnO NPs	42.5	21.25	0.414	1.233

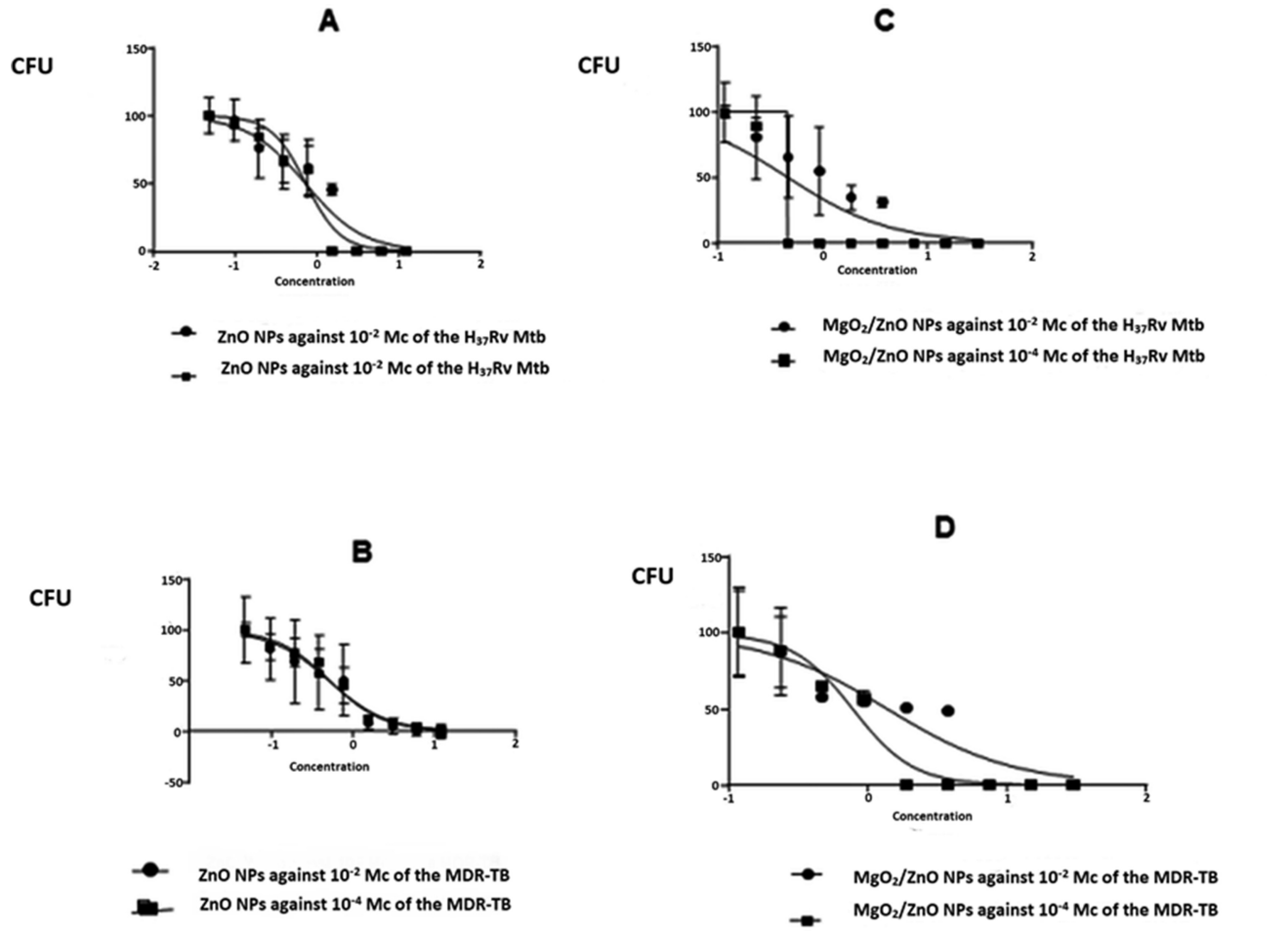

Fig. 1 – The dose–response curves of ZnONPs against *H_{37}Rv Mtb* (a) and against *MDR-Mtb* (b). The dose–response curves of MgONPs-ZnONPs against *H_{37}Rv Mtb* (c) and against *MDR-Mtb* (d) in 10^{-2} and 10^{-4} McFarland.

MICs was defined as the lowest drug concentration which prevented a color change from blue to pink.[9]

To determine the minimum bactericidal concentration (MBCs) of NPs against MDR-Mtb (Razi serum and vaccine research institute, IRAN), proportion method was also done. 10 mL of melted löwenstein-Jensen (LJ) agar and 10 mL of NPs were poured and subsequently, serial dilution was performed. On the following day, 100 μL of 10–2 and 10–4 McFarland of bacteria were added and then they are incubated at 37 °C. The colony-forming unit (CFU) of bacteria was counted after 28 days. The atomic force microscopy (AFM) (Institute for color science & technology, IRAN) of mixture MgONPs-ZnONPs fulfilled by commercial AFM system (Nanosurf, Switzerland).[10] The TEM image was prepared at the Institute of Biochemistry and Biophysics (IBB) of Tehran University.

2.4. *MTT assay*

To MTT assay, Vero and $HepG_2$ cell lines (Institute for tuberculosis research, the University of Illinois at Chicago, USA) (1 × 104 cells per well) were seeded in 96-well plates containing 100 μL of DMEM, separately.[11] 100 μL of MgONPs, ZnONPs, and MgONPs-ZnONPs were inoculated to each well. Next, 5 mg MTT per mL added. Cell viability calculated using DMSO treated Vero and $HepG_2$ as the 100% viable control and measured (A540 of NPs × treated sample/A540 of control) × 100.

2.5. *Statistical analysis*

Statistical analyses were prepared by using of Kruskal–Wallis test and Mann–Whitney U test. The statistical significance threshold was resolute as P-value ≤ 0.05.

3. Results and discussion

The MIC of MgONPs was 0.195 μg mL^{-1} against 10^4 of H_{37}Rv Mtb (Table 1A). According to the results, the MIC of ZnONPs was reported 0.937 μg mL^{-1} for 10^{-2} H_{37}Rv Mtb and the MIC of ZnONPs reached to 0.468 μg mL^{-1} for 10^{-4} H_{37}Rv Mtb. As well, 0.332 μg mL^{-1} and 0.166 μg mL^{-1} of MgONPs-ZnONPs was able

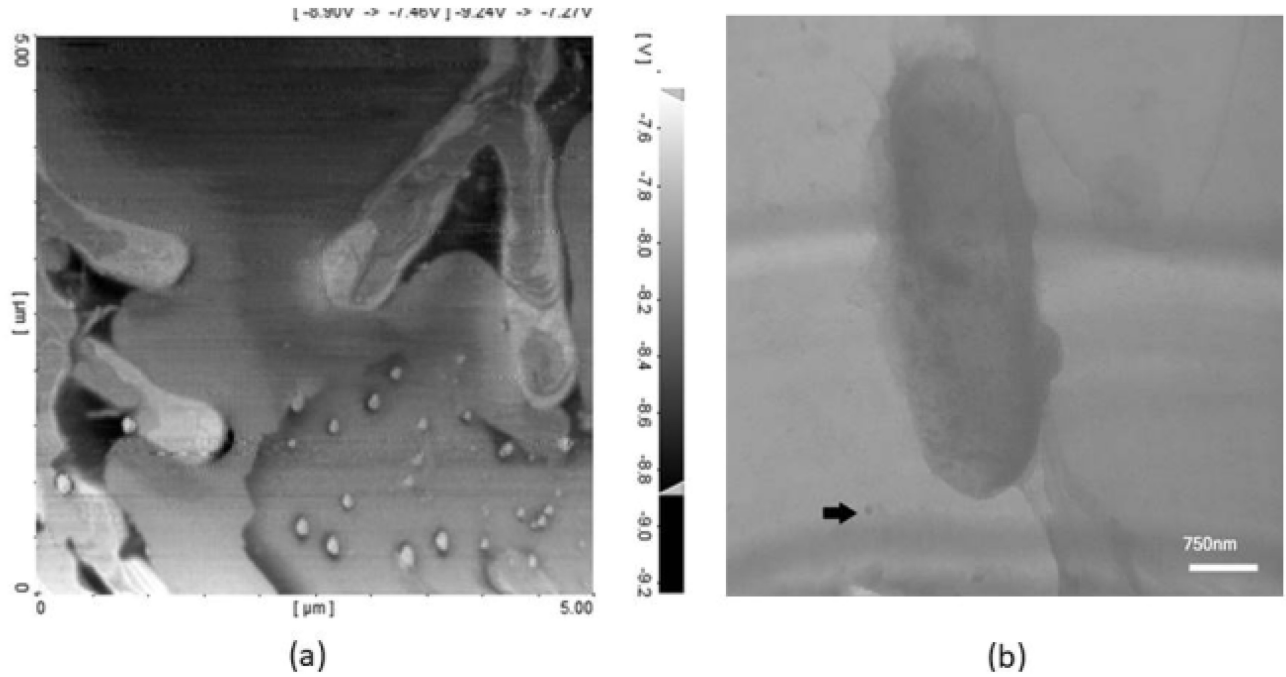

Fig. 2 – AFM image of *H_{37}Rv Mtb* and mixed MgONPs-ZnONPs (a) on silicon surface and TEM image (b) of NPs around of H_{37}Rv *Mtb* on the scale of 750 nm.

to inhibit 10^{-2} and 10^{-4} H_{37}Rv Mtb, respectively (Table 1A). The MIC of MgONPs against both 10^2 and 10^4 concentrations of MDR-Mtb were 12.5 μg mL^{-1} (Table 1A). The MIC of MgONPs/ZnONPs against 10^2 concentrations of MDR-Mtb was determined 0.166 μg mL^{-1} (Table 1A). The MIC of MgONPs/ZnONPs against 10^4 concentrations of MDR-Mtb reached to 0.664 μg mL^{-1} (Table 1A).

The MBCs of MgONPs against 10^2 and 10^4 concentrations of H_{37}Rv Mtb were 3.125 μg mL^{-1} and 1.875 μg mL^{-1}. The MBCs of MgONPs against 10^2 and 10^4 concentrations of MDR-Mtb were $\geq$12.5 μg mL^{-1} (Table 1A). The MBC of ZnONPs was 0.937 μg mL^{-1} against 10^4 concentrations of H_{37}Rv Mtb (Table 1A). Based on the results, the MBC value of ZnONPs increased to 1.875 μg mL^{-1} against 10^{-4} concentrations of MDR-Mtb. On the other hands, testing showed that the MBCs of MgONPs/ZnONPs reached to 1.328 μg mL^{-1} against 10^4 concentrations of both H_{37}Rv Mtb and MDR-Mtb (Table 1A).

Dose–response curves showed ZnONPs and MgONPs-ZnONPs have high potency to eliminate 10^{-4} MDR-Mtb (Fig. 1).

AFM image confirms that the MgONPs-ZnONPs are monodisperse (Fig. 2a). The sizes of the NPs were estimated at less than 50 nm. As we show in Fig. 2a, some of NPs attached to the cell wall of Mtb (Fig. 2a). It showed that agglomerated metallic NPs are able to attach to the cell wall and penetrates onto Mtb. The oval-shaped Mtb in the present of MgONPs-ZnONPs was demonstrated by TEM images (See also Fig. 2b).

MTT assay results showed that MgONPs inhibited the growth of $HepG_2$ cell lines (Table 1B). Also, 3.579 μg mL^{-1} of ZnONPs was able to inhibit the growth of $HepG_2$ and Vero cell lines. The results showed that the toxicity of mixture MgONPs-ZnONPs reached to 1.233 μg mL^{-1} against Vero cells. The results indicated the IC_{50} of $HepG_2$ cell lines in exposure to MgONPs-ZnONPs reached to 0.414 μg mL^{-1}.

The current study is the first experimental study of which has evaluated the MICs and MBCs of ZnONPs, MgONPs, and MgONPs-ZnONPs against H_{37}Rv Mtb and the MDR-Mtb. In this study, the toxicity effects of the NPs have evaluated, as well. This study reported the MIC of MgONPs against MDR-Mtb was 12.5 μg mL^{-1} (Table 1A). Up to now, there has not been the report in the light of antibacterial effects of MgONPs against *M. tuberculosis*. Previously, Nhu-YThi Nguyen has been reported that MgONPs was able to inhibit gram-positive, gram-negative, and endospore-forming bacteria.[3] Punjabi reported that 1.25 μg mL^{-1} of ZnONPs inhibited clinical isolated of MDR-TB. 4 Patil et al, reported that ZnONPs has ability to inhibit Mtb at 12.5 μg mL^{-1}.[12] We showed that the ZnONPs has the ability to eliminate MDR-TB at 1.875 μg mL^{-1} (Table 1A). Mixture MnO2NPs-ZnONPs also showed bactericidal effects on MDR-TB at 1.328 μg mL^{-1} the dose–response curves also showed that anti-tubercular impact of NPs depends on the concentration of MDR-TB (Fig. 1).

4. Conclusions

The size of NPs may also affect their anti-tubercular properties. The previous study showed that the ZnONPs with smaller size exhibited higher efficiencies against Mtb.[13] The bigger size of MgONPs -about 20 nm–in comparison with ZnONPs -about 4 nm-might be effective on weakly anti-tubercular activities of MgONPs. Moreover, researchers believed that metallic NPs have a strong tendency to agglomerate in the broth suspension because of their high surface energy, which could have affected the interactions and penetrations of these nanoparticles with Mtb.

4.1. *Limitations*

We believe that there are no standards of regular experimental techniques for testing the anti-tubercular properties of NPs. In fact, NPs with varying sizes, physicochemical characterizes, concentrations, and different initial seeding densities have been used in the previous studies, which do affect the results, significantly. We found that ZnONPs and mixture MgONPs-ZnONPs not only have higher bactericide behavior but might have also synergistic effects against MDR-TB. Actually, even though the current study does prove that MgO and ZnONPs have anti-tubercular properties against MDR-Mtb, the anti-tubercular potency of mixture MgONPs/ZnONPs is still unknown and incomparable.

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Conflicts of interest

All authors have none to declare.

Ethics approval

This study was in accordance with the declaration of Helsinki and an ethical approval was sought from the institutional Ethics Committee of Guilan University of Medical Sciences (Approval No. IR. GUMS.REC.1396.481).

Consent to participate

Because we only used leftovers from clinical specimens, the local ethics committee waived the need for informed consent.

Consent for publication

Not applicable. The preprint of the manuscript is already publicly available on the web (https://www.researchsquare.com/article/rs-4250/v1). The manuscript has not been formally published - it has just been posted as a preprint, so it is still eligible to be published in a journal.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Author's contributions

T. Yaghubi kalurazi and A. Jafari: conceived the study. T. Yaghubi kalurazi and A. Jafari: participated in the design of the study and performed the statistical analysis. A. Jafari: interpreted the data. A. Jafari: obtained ethical clearance and permission for study. A. Jafari: Supervised data collectors. A. Jafari: Drafting the article or revisiting it critically for important intellectual content. T. Yaghubi kalurazi: was project leaders and primary investigators of the study. All authors read and approved the final manuscript.

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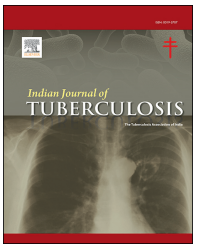

Original Article

Haematological profiles after Intensive phase of Anti Koch Treatment with special emphasis on bone marrow changes

*Debjani Basu, Tutor, Saumitra Biswas, Professor, Rudranarayan Ray, Assistant Professor**

Dept. of Pathology, BSMCH, Kenduadihi, Bankura, 722102, West Bengal, India

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ABSTRACT

Background: Tuberculosis remains a major public health problem in various parts of the world. It leads to various haematological changes. Study of these haematological changes will help better patient management.

Objective & methods: It is to evaluate haematological changes in tuberculosis patients and compare the result with special emphasis to bone marrow changes as active case search is sharply decreasing the miliary tuberculosis. It is also to evaluate the patients with before and after the Intensive Phase of Anti Koch Treatment. Sputum positive and sputum negative tuberculosis patients confirmed by other ancillary techniques were included into this study. It is conducted at a tertiary level hospital in rural area.

Result: In this study bone marrow hypercellularity was of erythroid series with only 1.92% patients showed granuloma in bone marrow aspiration. In addition to bone marrow changes, significant changes were evident in haemoglobin level, Erythrocyte Sedimentation Rate (ESR) Total White Blood Cell count and RBC count.

Discussion: In majority cases this study showed Erythroid Hyperplasia. It is sharp contrast with other study where myeloid hyperplasia was evident. This study also differs from other study where high number of bone marrow granuloma was reported. In this study only 1.92% cases showed bone marrow granuloma. This study also documented higher number of anaemic cases mostly because of the institute serves poor and tribal population.

Conclusion: In our study the cases showing granuloma and hyperplasia of myeloid series were limited. With introduction of Directly Observed Treatment and house to house active case search helped to sharply decrease bone marrow granuloma by limiting multi-organ spread. This study showed, ESR level may be considered as prognostic parameters of tuberculosis.

* *Corresponding author.*
E-mail address: rudranray@rediffmail.com (R. Ray).

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1. Introduction and background

Tuberculosis is a chronic granulomatous disease and a major public health problem in developing countries. India is the largest tuberculosis burden country, accounting for one - fifth of global incidence.[1] The Revised National Tuberculosis Control Programme (RNTCP), formulated in 1993, was based on WHO recommended Directly Observed Therapy (DOTS) strategy.[1,2] Tuberculosis causes various changes in haematological profile. But it is not highlighted in RNTCP management guidelines. This study aimed at changes that will help better patient management.

2. Objectives & methods

It is to evaluate haematological changes before starting treatment with Intensive Phase (IP) of anti-tubercular drugs (ATD-DOTS) and after taking and completion of IP of ATD-DOTS treatment. Both new and relapse cases, attending Department of Pathology, referred from Department of Chest Medicine (outdoor and indoor), were included in the study and patients suffering from other co-morbidities like diabetes mellitus, chronic renal failure, malignancy, haematological disorders, long standing corticosteroid therapy, pregnancy, lactation, trauma causing blood loss, old treated tuberculosis patients, HIV infected and drug resistant cases were excluded. Haematological and clinico-demographic data collected over 1 year were statistically analysed using IBM Statistical Package for Social Sciences software for Window Version 15.0. Normality of data were tested by using Kolmogorov–Smirnov, Shapiro–Wilk, Skewness, Kurtosis and Q–Q Plot & Histogram. Data analysed using Chi square test for nominal data and paired t-test, Wilcoxon signed rank test, one way ANOVA, Kruskal–Wallis Rankstest for continuous data, as applicable.

3. Results

A total of 52 patients were selected according to selection criteria. The distributions of clinical and demographic variables are shown in Table 1 and description [Mean ± Standard Deviation (SD)] of different haematological parameters are shown in Table 2.

Out of total 52 patients, males were 26 and female were 26 in number. Clinically among them pulmonary tuberculosis reported in 14 cases, pleural effusion in 22 cases, lymphadenopathy among six cases, miliary tuberculosis eight cases, Scrofuloderma in one cases and disseminated tuberculosis was in one case.

In the case of normally distributed continuous data like Erythrocyte Sedimentation Rate (ESR), RBC, WBC, paired t-test and one way ANOVA were done and in other variables which were found to be not normally distributed, Wilcoxon signed rank test & Kruskal–Wallis tests were applied to find any significant difference. In case of qualitative data, chi square test was done.

Paired sample t-test showed ESR: $t(51) = 10.261$, $p < 0.001$, Neutrophil: $t(51) = 3.619$, $p < 0.01$, and RBC: $t(51) = 9.432$, $p < 0.001$. These results indicated that there were significant differences and thus improvement of haematological parameters following IP of DOTs-ATD.

The Wilcoxon signed rank test showed that the observed differences of haematological parameters following IP of DOTs-ATD were present in case of Hb% (Z-6.275, $p < 0.001$) and total WBC count (Z-3.168, $p < 0.01$). Thus, the result rejected the null hypothesis.

4. Discussion

In the present study Pre-treatment bone marrow was hypercellular in 42.31% of patients and post-treatment bone marrow was hyper-cellular in 40.31% of patients. Micronormoblastic maturation was in 34.61% of patients and post-treatment bone marrow maturation showed micronormoblastic maturation in 32.69% of patients indicating a decrease in micro-normoblastic maturation. Among pre-treatment and post treatment cases bone marrow showed normoblastic maturation in 65.38% and 67.31% cases respectively. This study is contrast with the study of Lombard EH et al[3] and Knox- Macaulay HH et al[4] who have documented myeloid hyperplasia.

The most possible reason behind erythroid hyperplasia may be nutritional anaemia as the medical college serves tribal population and majority below poverty line.

The significant shift from the study of Samuelsson SM[5] and Campo E et al[6] has been seen in case of bone marrow granuloma where even up to 50%–100% miliary TB cases showed

Table 1 – Demographic and clinical parameters of the patients.

Age in years	Mean age 36.58 ± 12.816 (SD) in the range between 18 and 75 years
Gender	Male, 26 (50%); Female, 26 (50%)
Clinical diagnosis	Pleural Effusion, 22 (42.31%); Pulmonary TB, 14. [Sputum +ve, 6 (11.5%); –ve 8 (15.4%)] Tubercular lymphadenopathy 6 (11.5%) Miliary Tuberculosis, 8 (15.4%), Scrofuloderma, 1 (1.9%) Disseminated Tuberculosis, 1 (1.9%)
Treatment type	Cat-1, 50 (96.15%), Cat-II, 2 (3.85%)

Table 2 – Pre-IP and post-IP haematological values.

	Pulmonary TB (Mean ± SD)		Extra-pulmonary TB (Mean ± SD)		Others (Mean ± SD)	
	Pre-treatment	Post treatment	Pre-treatment	Post treatment	Pre-treatment	Post treatment
Hb%	9.97 ± 1.65 (SD)	10.96+-1.62	10.69 ± 2024	10.80+-2.07	10.04 ± 2.35	10.70+-2.07
ESR	100.94 ± 16.03	72.81+-19.49	80.77 ± 27.47	32.90+-19.31	85.78 ± 28.09	61.57+-23.35
TC-WB	11818.75 ± 7843.83	8612.50+-2080.98	10,650 ± 7793.39	8581.13+-2609.93	7978.57 ± 4200.77	7442.86+-2013.31

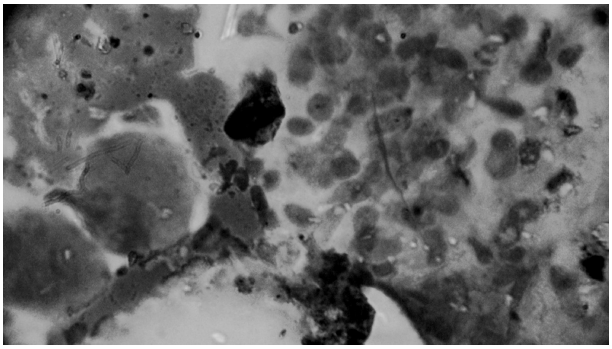

Fig. 1 – Well formed epithelioid granuloma in the bone marrow adjacent to macrophages.

bone marrow granuloma but in the present study bone marrow granuloma (Fig. 1) was evident only in 1.92% cases. This may be due to awareness programme created by Government institutions through television, radio and field workers who go door to door for active case detection that has facilitated early case detection and prompt treatment.

In previous studies, moderate degree of anaemia has been observed in 52%–72% of patients in most series.[7–21] Even without iron supplementation, ATD treatment resulted in correction of anaemia.[22] In present study, anaemia was present in 82.69% of patients before treatment and in 80.77% of patients after treatment with IP- ATD for 2 months, without iron supplementation. The number of cases showing anaemia was higher in the study that of the others who have showed about 52%–72% cases. The medical college serve among tribal population in majority so pre existing anaemia may be a reason for high number of anaemic cases.

In this study, pre-treatment RBC count was low in 76.92% of patients and post-treatment RBC count remained low in 51.92% of patients. Result shows there was a statistically significant increase and thus improvement in mean RBC count following IP-ATD from 3.45 (± SD 0.68) million/cu.mm to 4.00 (± SD 0.72) million/cu.mm ($p < 0.001$), an improvement of 0.55 million/cu.mm. These findings are similar to findings by Rohini, K et al,[20] Baynes RD et al[23] and Cartwright GE[24] and dissimilar to study of Kassa E,[19] where RBC count was significantly lowered after treatment. Improved nutrition of patients and response to treatment could possibly be reasons for improvement in this study, as this study was conducted in a medical college scattering majority of poor and tribal population, where a good number of patients might have been anaemic before having TB.

In present study, Leucocytosis was documented in 44.23% of patients and leucopenia in 1.9% of patients before treatment. After treatment proportion of patients with leucocytosis came down to11.54% and leucopenia remained the same in 1.9%of patients. Result shows statistically significant lowering and thus improvement in mean total WBC count following IP-ATD ($p < 0.01$).Thus, this study is similar to the other studies,[7,18,25–27] but unlike study of Olaniyi JA et al,[28] which did not show statistically significant ($p > 0.05$)difference in mean total WBC count between pre and post treatment patients.

In study of K Kaur, ESR value was found to be significantly raised in the tuberculosis patients, being mean ESR 66.86 mm/1st.hr.[29] The values of ESR in pulmonary tuberculosis were significantly decreased after treatment with anti-tubercular drugs, ($p < 0.001$) and indicate good response to treatment and a good prognosis.[30–32]

In present study, ESR, before treatment, was raised in 100% cases and after treatment, was raised in 90.38% cases. Result shows statistically significant ($p < 0.001$) difference between mean ESR before and after treatment indicating a lowering of ESR and improvement with treatment following intensive phase of DOTs-ATD from 88.33 ± 25.76 to 50.13 ± 5.48 ($p < 0.001$); an improvement of 38.20 ± 20.28 mm. 1st hr. Thus, present study is similar to other studies on ESR.[29–32]

In this study no case showed Acid Fast Bacilli in bone marrow.

No adverse side effects of IP-ATD on haematological profiles in TB patients were noted. Thus, present study is similar to other studies.[33]

5. Conclusion

This study enumerates the fact that with access of prompt Anti Koch Treatment and early diagnosis lead to striking improvement of haematological parameters. Parameters like ESR though is a non specific diagnostic tool but can be used as an indicator in terms of response to the treatment which is very important for developing countries as resources are low and costly diagnostic tools are only available in the tertiary care centres. After all tuberculosis does target the poor. This study also shows that contrast to other studies that miliary cases are less and formation of bone granuloma are also very low.

Consent

Consent of the patients were taken by Department of Chest Medicine for procedure and Ethical committee of B.S.M.C.

Declaration of competing interest

All authors have none to declare.

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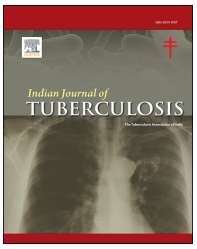

Original Article

Diagnostic yield of semi rigid thoracoscopy in unexplained exudative pleural effusion- Experience from tertiary care hospital of east India

Deependra Kumar Rai, Additional Professor & Head [a,*], *Laxmi Niwas Niwari, Senior Resident* [a], *Saurabh Karmakar, Associate Professor* [a], *Saket Sharma, Consultant* [b]

[a] *Department of Pulmonary Medicine, AIIMS, Patna, 801505, India*
[b] *Consultant, Pulmonary Medicine, Asian Hospital, Patna, India*

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ABSTRACT

Introduction: In most of the pleural effusion, fluid analysis generally gives the etiological diagnosis but in almost 20% it remains unclear. This study was designed to determine the diagnostic yield of a pleural biopsy using semi rigid thoracoscope and its complication rates.

Materials and methods: This was a retrospective observational study conducted in the Department of Pulmonary Medicine, AIIMS Patna. All the patients diagnosed as unexplained pleural effusion between Jan 2018 and December 2019 were included in the study.

Results: Total 76 out of 97 patients with unexplained exudative pleural effusion underwent medical thoracoscopy in the given period of 2 years. The mean age of the patients was 57.63 years. There were 46 males and 30 females. 38 patients (50%) had right-sided pleural effusion. More than half (52.6%) of study patients were on Anti-tubercular treatment in which only 11.84% had tuberculosis. In both unilateral and bilateral pleural effusion, the proportions of small, moderate, and large size of pleural effusions were 10.52, 42.10, and 47.36%, respectively. Thoracoscopy yielded a definitive diagnosis in 66 out of 76 patients (86.84%), and in 10 patients (13.15%), biopsy was inconclusive. Of 76 patients, malignancy was confirmed in 58 (76.31%), and tuberculosis in 8 (11.84%) patients

Conclusion: This study concludes that, medical thoracoscopy with semi-rigid thoracoscope is an invaluable tool in the diagnosis of patients with unexplained exudative pleural effusion. It is a very simple and safe method with high diagnostic yield and associated with few complications. Malignancy was found to be the most common cause of unexplained exudative pleural effusion

* *Corresponding author*. +917764981421 (mobile).
E-mail address: deependra78@gmail.com (D.K. Rai).

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1. Introduction

Medical thoracoscopy is an endoscopic procedure by which we visualise pleural cavity. It is a minimally invasive procedure that was first developed by Hans Christian Jacobeus, a Swedish internist[1] in 1910, who is also called "father of thoracoscopy." In old era, it was mainly used in the treatment of pulmonary tuberculosis (TB) and tubercular pleural adhesions, but in last few years, thoracoscopy has gained a lot of interest and popularity among chest specialist mainly in etiological diagnosis of pleural effusions.[2]

The major indication for medical thoracoscopy is evaluation of exudative pleural effusions which remain unexplained after pleural fluid analysis, where thoracoscopy is suggested as an alternative to closed pleural biopsy.

In all the patients with pleural effusion, cytobiochemical and microbiological analysis of pleural fluid is needed to establish the aetiology; however, it is useful for diagnosis only in up to 60% of cases[3] and in around 20% of the cases, aetiology often remains unclear even after extensive diagnostic workup.[4]

According to currently available literature, the diagnostic sensitivity of medical thoracoscopy using rigid thoracoscopy ranges from 85% to 93%.[5] A recently published Meta analyses have reported that the sensitivity and specificity of semi-rigid thoracoscopy are 91% and 100%, respectively,[6] with efficacy almost close to surgical thoracoscopy.

In Indian scenario, there are fewer studies that have been done on the role of thoracoscopy in cases of undiagnosed pleural effusion.[7–10] This study was designed to find the diagnostic yield of a pleural biopsy using semi rigid thoracoscope and its associated complication rates.

2. Materials & methods

2.1. *Study design: Retrospective observational study*

This was a retrospective observational study conducted in the Department of Pulmonary Medicine, AIIMS Patna. All the patients with unexplained pleural effusion between Jan 2018 and December 2019 were included in the study.

Unexplained pleural effusion was defined as an effusion which remains undiagnosed by initial pleural fluid analysis including pleural fluid adenosine deaminase (ADA) estimation and at minimum three pleural fluid analyses negative for malignant cells.

The size of a pleural effusion was categorised into small, moderate, or large based on CT imaging according to the methods described by Moy and colleagues[10]

Inclusion criteria

1. All the patient diagnosed as unexplained pleural effusion

Exclusion criteria

1. Patient unwillingness for procedure
2. Contraindication for thoracoscopy

All study patients underwent detailed history and workup include Pleural fluid analysis, CT chest and blood investigation such as complete blood count, coagulation profile include prothrombin time (PT), activated plasma thrombin time (aPTT) and platelet count to rule out bleeding diathesis. Platelet count less than 75,000/mm3 and those patients with PT or aPTT increased by more than four seconds above control were used as exclusion criteria for thoracoscopy. Other contraindications for thoracoscopy like patient not giving consent for procedure, minimal pleural effusion, haemodynamic instability, arrhythmias and intractable cough.

Sociodemographic characteristics of the patient including the age, gender, clinical symptoms, pleural fluid analysis, that includes total and differential count, protein, LDH and glucose values, ADA levels, stain for acid-fast bacilli (AFB) and cytology findings and findings of the CT of the chest were recorded. Data are presented in a descriptive manner.

Procedure details: Medical thoracoscopy was performed with semirigid thoracoscope (Olympus LTF-160) under conscious sedation in the endoscopy suite. Written informed consent was taken from each patient or close relative before the procedure. Intravenous line was placed by nursing staff in the upper limb opposite to the side of thoracoscopy. Patient's vital parameters include electrocardiogram, pulse, blood pressure, and oxygen saturation were continuously monitored throughout the procedure. All patients received oxygen by nasal cannula and were placed in the lateral decubitus position with the ipsilateral arm abducted and elevated over the head to maximize access to the hemithorax. After positioning of patient, thoracic ultrasound was performed to assess the ideal entry site for the trocar. Procedure was performed under conscious sedation, 2 mg Midazolam was administered before commencing the procedure. Local anaesthesia lignocaine was administered locally, and a 2–3 cm long incision was made parallel to the upper margin of the rib to avoid damage to neurovascular bundle. After incision, blunt dissection was performed using a straight and curved artery forcep in the chest wall as far as the parietal pleura. After penetration of parietal wall, a trocar was introduced and the semirigid thoracoscope was inserted through the trocar. Generally 2–6 biopsies of the abnormal lesion inside the pleural cavity were taken by biopsy forceps. If no gross abnormalities were visible on parietal pleura, multiple biopsies were taken from different area. Upon completion of the procedure, a chest tube was inserted through the trocar, and the trocar was removed.

2.2. *Statistical analysis*

Continuous data are presented as mean ± SD and categorical variables in relative frequencies, and percentages. All statistical analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results

Total 97 patients had unexplained exudative pleural effusion in given period of 2 years. Medical thoracoscopy was performed in 76 patients as 21 patients were excluded due to less

pleural fluid, extensive loculation or other complication. The Characteristics of the patients are summarised in Table 1.

The mean age of the patients was 57.63 years. There were 46 males and 30 females. 38 patients (50%) had right-sided pleural effusion. More than half (52.63%) of study patients were on anti-tubercular treatment. Large number of patients (71.05%) had therapeutic pleural fluid aspiration (at least 500 ml) atleast twice before being subjected for thoracoscopy. In both unilateral and bilateral pleural effusion, the proportions of small, moderate, and large size of pleural effusions were 10.52, 42.10, and 47.36%, respectively. The appearance of pleural effusion was red in 76.31% of patients, and was straw coloured in 23.68%.

In the study patients, we found one or more abnormalities on the surface of parietal or/and visceral pleura during medical thoracoscopy. As shown in Table 2, pleural nodules, redness, pleural adhesion, and the other pleural pathological changes were observed (Figs. 1–3).

Thoracoscopy yielded a definitive diagnosis in 66 (86.84%) out of 76 patients, and in 10 patients (13.15%), biopsy was inconclusive. Of 76 patients, malignancy was confirmed in 58 (76.31%), and tuberculosis in 8 (11.84%) patients (Table 3).

Table 4 summarises finding of various studies with present study.

4. Discussion

Pleural effusion is commonly encountered by pulmonologist. In most of the patients, aetiology is diagnosed by cyto-biochemical analysis but in almost one fifth patient, aetiology remain unexplained.[4] In this scenario pleural biopsy is required for histological confirmation. As percutaneous blind needle pleural biopsy is having low sensitivity even in expert hands,[10] thoracoscopy is preferred as it provides diagnosis in most of the pleural effusion patients in whom the diagnosis had not been achieved by conventional investigations. Thoracoscopy has advantage in comparison to blind pleural biopsy as it gives an opportunity to perform biopsy from suspicious pleural lesions under direct vision. It also provides better

Table 1 – Baseline characteristics of study patients.

	Total (n = 76)
Age (years)	57.63 ± 14.81
Sex (M: F)	46:30
Smoker/Exsmoker/Nonsmoker	18/14/46
TDI (Total duration of illness in month)	07 (1–60)
History of Antitubercular treatment	40 (52.63)
History of at least 2 therapeutic pleural taps (>500ml)	54 (71.05)
Side of Pleural effusion	
Right	38 (50)
Left	32 (42.10)
Bilateral	06 (7.89)
Size of effusion	
Small	08 (10.52)
Moderate	32 (42.10)
Large	36 (47.36)
Colour of effusion	
Red	58 (76.31)
Straw	18 (23.68)
Pleural effusion characteristics	
Total protein	4.56 ± 1.16
Glucose	85.98 ± 35.12
LDH	1215.26 ± 1083.59
ADA	22.60 ± 10.79

Table 2 – Thoracoscopic finding of study subjects.

Thoracoscopic appearance	N = 76
Redness	40 (52.63)
Nodule	60 (78.94)
Adhesion	12 (15.78)
Other	10 (13.15)
Average duration of Chest tube (Days)	16.3 ± 6.80

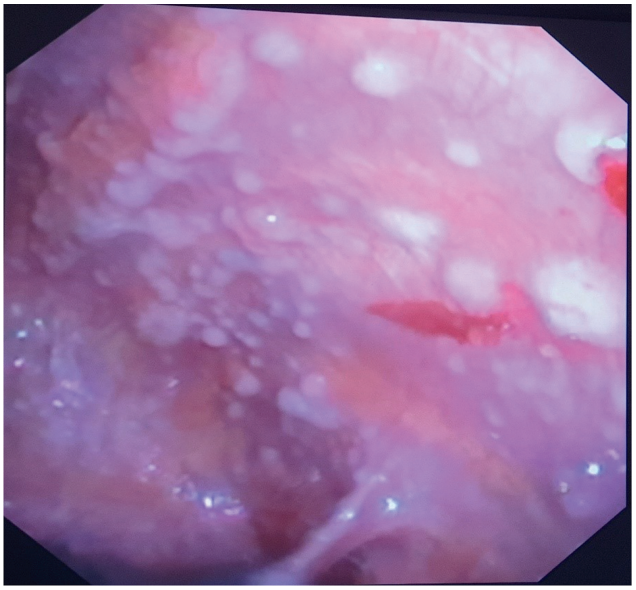

Fig. 1 – White nodular lesion (sago grain) which come out as tuberculosis.

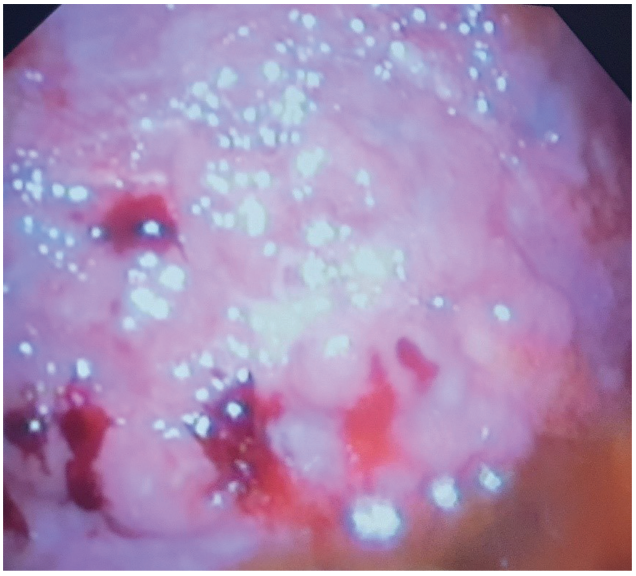

Fig. 2 – Nodular growth which bleed on touch diagnosed as metastatic adenocarcinoma.

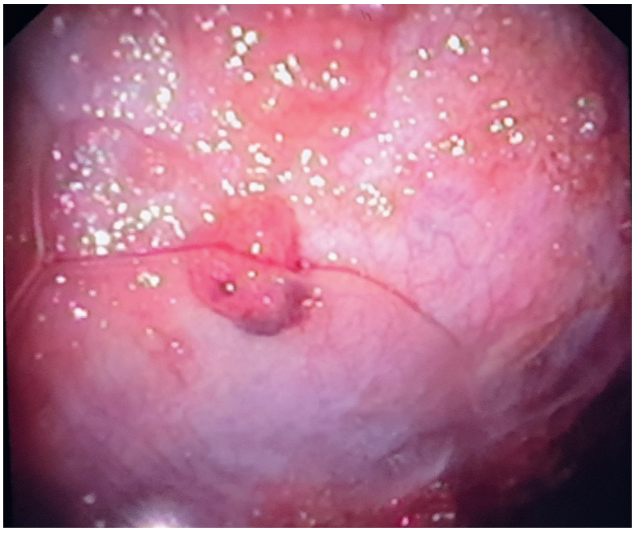

Fig. 3 – Multiple nodular lesion on parietal growth in adenocarcinoma lung.

Table 3 – Histopathological diagnosis from thoracoscopic biopsy (n = 76).

Histopathological diagnosis		N = 76 (100%)
Malignancy		58 (76.31)
	Adenocarcinoma lung	22 (37.93)
	Poor differentiated carcinoma	13 (17.10)
	Squamous cell carcinoma	04 (6.89)
	Metastatic carcinoma	08 (13.79)
	Mesothelioma	07 (12.06)
	Large cell Neuroendocrine tumour	02 (3.44)
	Lymphoma	02 (3.44))
Tuberculosis		08 (10.52)
Inconclusive		10 (13.15)

visualisation of loculated pleural effusions because of the ability to break down the loculi, either with diathermy or with biopsy forceps.

More than half (52.6%) of study patients were on Anti-tubercular treatment but only 11.84% patient had tuberculosis confirmed histologically and this could be the cause for delayed presentation. Other studies showing higher proportion of tuberculosis could be due to difference in inclusion criteria. Mootha et al[7] found tuberculosis in 22.85% of 35 study patients. Prabhu et al[9] and Wu Yb et al[13] showed 23.52 and 24.03% of study patients have tuberculosis. In one patient, pleural biopsy genexpert showed Multi drug resistance tuberculosis.

Large number of patients (71.05%) underwent therapeutic pleural fluid aspiration before being subjected for thoracoscopy.

Half of study patients have right sided pleural effusion while 7.5% have bilateral effusion. Prabhu et al[9] and Wu Yb et al[13] showed 33.9% and 43.6% patients have right sided effusion. CB Patil et al[12] found right-sided, left sided and bilateral pleural effusion in 52.7%), 32.5%, and 14.7% respectively.

The overall diagnostic yield of thoracoscopic pleural biopsy in the study was 66/76 (86.84%). In other studies, yield varies from minimum (26/35) 74.3%[7] to as high as (91/95) 95.8%.[8] Hucker et al[2] from England reported a diagnostic sensitivity of 80.3% in their study which included 102 patients, and Hansen et al[11] from Denmark were able to achieve diagnosis in 90.4% in a total of 147 patients of undiagnosed pleural effusion. Another Indian study showed diagnostic yield of 110/129 (85.3%) in undiagnosed pleural effusion.[12]

Malignancy was found in (58/76) 76.31% of patients with unexplained pleural effusion. Hucker et al[2] reported malignancy in 59% of cases, Mootha et al[7] reported 48.6%, Dhanya et al[8] reported 55.8%, and Hansen et al[11] reported malignancy in 62% of their study population. Wu Yb et al[13] found malignancy in 92.7% of their study patients and these high figure due to selection of only malignant pleural effusion patient for thoracoscopy.

Adenocarcinoma lung was most common malignancy followed by poorly differentiated carcinoma and metastatic carcinoma which in line with previous study.[12]

In our series, out of 76 patients, we did not found any major complication except 12 patients had prolong air leak and one patient developed re-expansion edema which recovered after non-invasive ventilator support.

5. Conclusion

This study concludes that, Medical thoracoscopy with semi-rigid thoracoscope is an important tool in the diagnosis of

Table 4 – Comparison of previous studies with present study.

Characteristics	Mootha et al[7](n-35)	Patil et al[12] (n-129)	Wu Yb et al[13] (n-342)	Prabhu et al[9](n-68	Present study (n-76)
Thoracoscope type	Rigid	Rigid	Rigid	Semirigid	Semi rigid
M:F	25:10	92:36	183/159	55:13	46:30
Age	48.68 ± 14.0	54 ± 20.16	62.8 ± 9.7	50.5	57.63 ± 14.81
Right/Left/Bilateral			43.6/38.9/17.5	33.9/66.1/0	50/42.1/7.8
Small/Moderate/Large			16.7/12.9/70.4		10.52/42.10/47.36
Haemorrhagic			55.9		76.31
Histopathological diagnosis					
Malignancy	51.42	56.6	92.7	31.57	76.31
Tuberculosis	22.85	24.03		23.52	11.84
Inconclusive	25	14.7	7.3	32.35	14.74

Number in table represent proportion except number of male/females which is actual number of patients.

patients with unexplained exudative pleural effusion. It is very simple and safe method with high diagnostic yield and low complication rates. Malignancy was found to be the most common cause of unexplained exudative pleural effusion. Empirical anti-tubercular treatment is important cause of delayed presentation and could be the cause of detection of malignancy in advance stage.

Conflicts of interest

All authors have none to declare.

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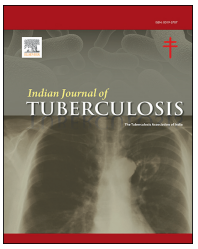

Original Article

Is the rise in Crohn's disease in India accompanied by a fall in intestinal tuberculosis? A single-center experience

Pavan Dhoble, Devendra Desai, Philip Abraham*

Division of Gastroenterology, P D Hinduja Hospital, Mumbai 400016, India

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ABSTRACT

Introduction: The relationship between the incidence of intestinal tuberculosis (TB) and Crohn's disease (CD) is interesting, especially considering the striking similarity between the two conditions. Some studies from Asian populations suggested that the incidence of intestinal TB decreases when there is an increase in CD.

Aim: To compare the incidence trend between intestinal TB and CD over 15 years.

Methods: Medical records of patients seen in the Division of Gastroenterology over 15 years (2005–2019) were reviewed. CD was diagnosed according to the Copenhagen criteria. Intestinal TB was diagnosed in the appropriate clinical situation if any one or more of the following was present: (1) positive TB MGIT culture; (2) positive Gene Xpert for TB; (3) suggestive histologic findings, with positive tissue acid-fast bacillus (AFB) on smear or with sustained response to anti-TB therapy. The incidence time trend of patients with CD and intestinal TB diagnosis was then studied year-wise.

Results: 632 medical case records were accessed; 60 patients were excluded due to inadequate data or not fulfilling diagnostic criteria. The 572 patients included 224 with intestinal TB (median age 37 years, IQR 22; 125 [56%] females) and 348 with CD (median age 40 years, IQR 25; 159 [46%] females [$p < 0.02$ as compared to TB]). Thus, more patients with CD were seen during the study period, but there was no correlation between the incidence of the two conditions ($r = 0.318$; $p = 0.25$).

Conclusion: In Indian patients in a single private-sector center, there was no inverse correlation between the incidence of intestinal TB and CD over 15 years.

* *Corresponding author*. Division of Gastroenterology, P D Hinduja Hospital and MRC, Mahim, Mumbai, 400016, India. Tel.: +91 22 24447106, fax: +91 22 24441425.

E-mail address: devendracdesai@gmail.com (D. Desai).

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1. Introduction

India accounts for about one-fourth of the global burden of tuberculosis (TB).[1,2] Intestinal TB accounts for 2%–6% of all patients with TB.[3,4]

Inflammatory bowel disease (IBD) is being increasingly diagnosed in India[5]; the estimated burden is 1.4 million, second only to that of the US.[6] The ratio of ulcerative colitis to Crohn's disease (CD) in India is about 1.82–2:1, with CD being increasingly recognized.[7–9] In a recent multicenter Asia–Pacific survey by Ng et al, the incidence of CD reported from Hyderabad, a southern Indian city, was 3.9/100,000[10]; CD is more common in southern India as compared to the northern parts.[9]

The striking similarity between intestinal TB and CD is well known, to the extent that no combination of tests (except culture) can universally distinguish the two conditions. The isolation and incrimination of mycobacteria in CD[11–14] increases the overlap between the two conditions. In fact, in countries endemic for TB, such as India, empiric treatment with anti-TB drugs when in doubt is accepted in national guidelines.[15]

The West has seen a reduction in TB and rise in IBD over decades. For example, the number of TB cases in Britain fell from 117,000 in 1913 to around 5000 in 1987.[16] There has been a steady rise in IBD since 1950.[17,18] The decrease in TB in the black population in the US was accompanied by a corresponding increase in CD.[19] In some Asian populations too, the incidence of TB is declining, with increase in incidence of IBD. Tsironi et al. reported that the incidence of IBD has increased and abdominal TB has fallen over the last decade in Bangladeshi immigrants in East London.[20] A similar inverse trend has been reported from Korea as well.[21]

The rising incidence and prevalence of CD in Asia has been variously attributed to host and environmental changes (diet, nutrition, hygiene, immunity),[22] in addition to increased awareness among local physicians.[5] If CD is indeed a result of altered body response to mycobacteria, a change in these factors should be reflected in an increase in CD with decrease in TB infection, at least in centers where the former phenomenon is being observed.

We studied the trend over the last 15 years in a center that has seen a reasonable number of patients with CD over this period.

2. Material and methods

This was a retrospective analysis of archived records at P D Hinduja Hospital, a tertiary-level private-sector hospital. Waiver-of-consent approval was given by the institutional review board (Project code: 1102-17-DD). Medical case records of patients seen in the Division of Gastroenterology as outpatients or inpatients over 15 years (2005–2019) were reviewed. Where records were incomplete, attempt was made to contact patients telephonically.

The diagnosis of intestinal CD was made according to the Copenhagen diagnostic criteria.[23] Intestinal TB was diagnosed if any one or more of the following was present in biopsy specimens (obtained at endoscopy or surgery) in the appropriate clinical setting: (1) positive TB MGIT culture; (2) positive Gene Xpert for tuberculosis; (3) suggestive histologic findings, with positive tissue acid-fast bacillus (AFB) smear, or with sustained clinical response to anti-TB therapy on 2-year post-treatment follow up ('clinically diagnosed case'[24]). Patients with inconclusive diagnosis were excluded.

Data were recorded in an Excel worksheet (Microsoft Corp. 2019) as number of patients with CD and intestinal TB listed year-wise. Chi-square test and Pearson's correlation coefficient were calculated on www.socscistatistics.com.

3. Results

A total of 632 case records were accessed; 60 patients were excluded from analysis due to inadequate data or not fulfilling diagnostic criteria. The 572 patients included 224 with intestinal TB and 348 with CD. Over 75% of patients had a definitive investigation report to support the diagnosis of intestinal TB; in the rest, the diagnosis was based on suggestive features and sustained response to anti-TB treatment (Table 1). The details of the latter group are shown in Table 2.

The median age of the patients with intestinal TB was 37 years (IQR 22); 125 (56%) were females. In the majority of patients, the disease location was within the reach of a colonoscope; in 29%, the diagnosis was aided by tissue samples from other locations (Table 2).

The median age of the patients with CD was similar, 40 years (IQR 25); 159 (46%) were females ($\chi 2 = 5.58$; $p < 0.02$ as compared to TB). Nearly one-half of patients with CD had ileocolonic involvement; the rest had ileal or colonic involvement (Table 3). Two-thirds had non-stricturing, non-penetrating disease (Table 3).

Fig. 1 shows that the incidence trend for the two conditions was similar ($r = 0.318$, $p = 0.25$; Pearson's correlation coefficient).

4. Discussion

The relationship between the incidence of intestinal TB and CD is interesting, especially considering the striking similarity between the two conditions. If the nutrition-hygiene-immunity hypothesis is indeed true, we would expect an

Table 1 – Diagnostic criteria for intestinal TB (n = 224) in appropriate setting.

Diagnostic criteria	No. of cases (%)
Histology and culture positive	65 (29%)
Only histology positive	64 (28.6%)
Response to anti-TB treatment	54 (24.1%)[a]
Only culture positive	30 (13.4%)
AFB smear positive	10 (4.5%)
Gene Xpert positive	3 (1.3%)

Values are presented as number (%).
Some patients had more than one criterion positive.
[a] See Table 2.

Table 2 – Supportive evidence in those diagnosed as intestinal TB with sustained clinical response to treatment (n = 54).

Evidence	No. of cases (%)
Clinical features	49 (90.7%)
Radiological features	43 (79.6%)
Colonoscopy features	35 (64.8%)
Diagnosed TB elsewhere[a]	16 (29.6%)

Values are presented as number (%).
[a] Ascites 5, lymph node 2, duodenum 1, renal 1, pulmonary 5, pleural 2, bone 1, brain 1, past history of pulmonary TB 3, positive interferon gamma release assay 2, positive Mantoux test 1.

Table 3 – Distribution of Crohn's disease location and behavior (n = 348).

Location	No. of cases (%)
Ileal (L1)	102 (29%)
Colonic (L2)	82 (24%)
Ileo-colonic (L3)	162 (47%)
Behavior	**No. of cases (%)**
B1 (Non-stricturing, non-penetrating)	228 (66%)
B2 (Stricturing)	83 (24%)
B3 (Penetrating)	29 (8%)
B2 and B3 (Both stricturing and penetrating)	8 (2%)

Values are presented as number (%).
Concomitant upper GI involvement (L4) was seen in 41 (12%) patients; isolated upper GI involvement was seen in only 2 (0.5%) patients. Concomitant perianal disease (p) was present in 60 (17%) patients.

inverse relationship in the relative incidence of these conditions. Data on Bangladeshi immigrants in East London from the Britain National Tuberculosis Program[20] and from Korea[21] suggest such a phenomenon. It is important to know whether such a trend exists not only because it suggests commonality but also because allocation of resources can be reviewed.

In our retrospective analysis of data over the 15-year period between 2005 and 2019, 224 patients with intestinal TB and 384 with CD were diagnosed and/or treated at our center. However, we did not observe any trend in the respective incidence of these two conditions, unlike the findings in the studies from East London and Korean.[20,21]

We encountered more patients with CD than with intestinal TB during the study period, although there was no rising trend during this period. There is a potential bias in these data because ours is a private-sector hospital; we suspect that the scene may be different in public-sector institutions that cater more to the lower socio-economic strata, and also in various regions of this heterogeneous country. Private hospitals currently provide about 80 percent of outpatient care and 60 percent of inpatient care in India.[25]

Table 4 shows the incidence of TB and CD in eight countries that have the highest burden of TB, together constituting two-thirds of the total TB burden in the world.[1,10,26,27] Among these countries, India has a higher incidence of CD. Is this due to increased genetic susceptibility to CD? Or is it a result of undefined changing environmental factors? Although India still meets many of the WHO indices conducive to TB,[2] the total TB cases has decreased (300/100,000 to 199/100,000) between the years 2000 and 2018.[1]

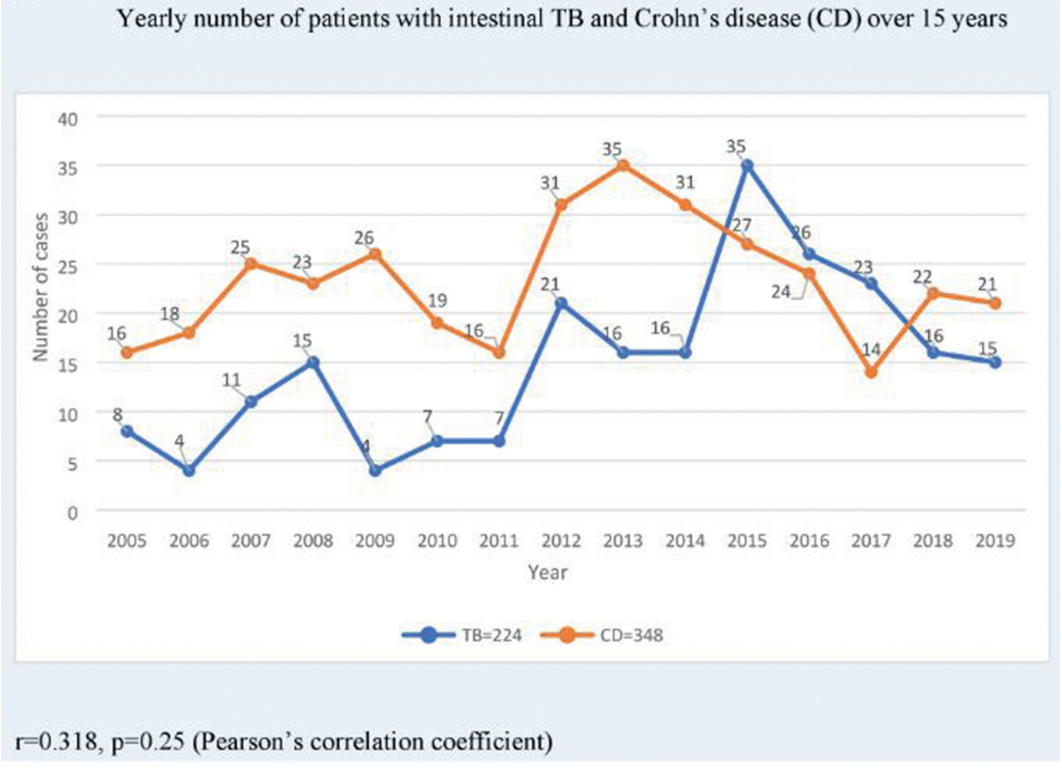

Fig. 1 – Yearly number of patients with intestinal TB and Crohn's disease (CD) over 15 years.

Table 4 – Incidence of TB and CD in 8 countries with highest burden of TB.[1,10,26,27]

Country	% of global burden	Incidence of TB (per 100,000)	Incidence of CD (per 100,000)
India	27	199	3.9
China	9	61	0.36 (pooled)
Indonesia	8	316	0.33
The Philippines	6	554	0.14
Pakistan	6	265	No data in natives
Nigeria	4	219	8 case reports
Bangladesh	4	221	No data in natives
South Africa	3	520	Blacks 0.3, Whites 2.6, Cape colored 1.8

Values are presented as number or %.

A quarter of our patients with TB had the diagnosis based on previously defined criteria for 'clinically diagnosed case'[24] of TB; this emphasizes the need for continued suspicion of TB in a high-prevalence country like India in patients with inconclusive features, and justifies the use of anti-TB treatment when in doubt. We used sustained clinical response to anti-TB therapy at 2-year follow up as a diagnostic criterion. In a recent study from India,[28] more than 90% of patients with intestinal TB, and up to 38% of patients with CD, responded initially to anti-TB treatment; however, at 1-year follow up, the patients with intestinal TB sustained their response, whereas up to 80% of patients with CD worsened. Multi-drug resistant tuberculosis (MDR-TB) would also behave similarly; however, studies from India and South Korea have shown very low prevalence (5.4% and 2.7%, respectively) of MDR-TB.[29,30]

In summary, we did not find inverse correlation between the incidence of intestinal TB and CD. Our study is limited by the fact that it is a single-center private-sector study from one region in India; but we believe that this should not affect an epidemiologic correlation between two diseases. The country may still be in the transition era between the two conditions; more studies from India are required to ascertain whether a time-trend will change with the passage of years.

Contributions from authors

All three authors have contributed to conception and design of the study, analysis and interpretation of the data, drafting the article or revising it critically for important intellectual content, and final approval of the version to be published, and accept accountability for all aspects of the work.

Statement of ethics

Study protocol was approved by the institutional review board (Project code: 1102-17-DD). Waiver-of-consent approval was given by the institutional review board.

Conflicts of interest

The authors have none to declare.

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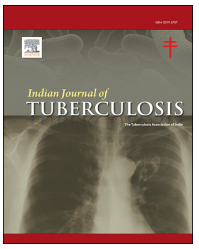

Original Article

Determinants of lymph node resolution in patients of tubercular lymphadenitis treated with anti tuberculous chemotherapy: A hospital based longitudinal study

Alkesh Kumar Khurana [a,*], *Vikas Gupta* [b], *Deepti Joshi* [c], *Abhishek Goyal* [a], *Ujjawal Khurana* [c], *Abhijit Pakhare* [d]

[a] *Pulmonary Medicine, AIIMS Bhopal, India*
[b] *Otorhinolaryngology & Head and Neck Surgery, AIIMS Bhopal, India*
[c] *Pathology & Lab Medicine, AIIMS Bhopal, India*
[d] *Community & Family Medicine, AIIMS Bhopal, India*

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ABSTRACT

Introduction: The variable course of illness in patients of Tubercular lymphadenitis remains a therapeutic challenge to treating physicians in a significant proportion of patients. This study was aimed to explore the possible determinants which could predict the outcome of this subgroup of patients.

Methodology: This was a prospective cohort study where 94 patients of TB lymphadenitis were enrolled who could be followed up till the end of treatment. They were evaluated in the beginning and monitored till the end of treatment keeping into account the clinical behaviour of lymph nodes during the course of Anti tubercular chemotherapy.

Results: Out of 94 patients, 60 had their lymph nodes resolved at the end of prescribed treatment duration wheras 34 were classified as partial responders. Another 26 amongst them had their nodes resolved by an extension of continuation phase by 3–6 months. Presence of bilateral and multiple lymph nodes, necrosis on Fine needle aspiration at initial diagnosis and occurrence of Paradoxical upgrading reaction were associated with the partial resolution of lymph nodes at the end of stipulated ATT duration.

Conclusion: Treatment duration should be individualized by the treating physicians. Certain parameters mentioned above can be taken as warning signals of patients ending up as partial responders and hence the need of a prolonged extension phase.

* *Corresponding author*. Tel.: +91-7773010091.
E-mail address: lungcancer@rediffmail.com (A.K. Khurana).

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1. Introduction

Tubercular Lymphadenitis is the most common extrapulmonary manifestation of tuberculosis, contributing to 30–40% of all extrapulmonary cases.[1,2] It remains as a therapeutic challenge to physicians because of inconsistent physical findings and investigation results.[3] Usually the diagnosis is made on Fine Needle Aspiration Cytology (FNAC) but some cases need biopsy for accurate histopathology picture.[2,4] The role of Polymerase Chain reaction (PCR) based assays have increased dramatically in recent times in the diagnosis of this particular disease.[3] Unpredictable treatment response adds to the woes of the treating physician. Concomitant HIV infections, Multi Drug Resistant (MDR) TB and infection by Atypical Mycobacteria add to the complex and varied presentation of Tubercular lymphadenitis patients.[2,5] In this study, we have tried to evaluate some hypothesised determinants responsible for varied resolution response of peripheral lymph nodes in these patients.

2. Materials and methods

Hospital based longitudinal study was carried out in the department of Pulmonary Medicine in collaboration with the department of Otorhinolaryngology and department of Pathology at our institute. Clearance was taken from institutional ethics committee and informed consent was obtained from patients before enrollment. Patients presenting with isolated peripheral lymphadenopathy with clinical suspicion of tuberculosis were provisionally included in the study. Final enrollment was done based on the diagnosis of tuberculosis which was made on a holistic approach which included FNAC/Histopathology findings/PCR based assays/clinical improvement with Anti tuberculous therapy (ATT). After written informed consent, initial assessment at the time of enrollment included following parameters:(a) Age and Gender (b) Site and number of lymph nodes (c) Size of lymph nodes whereby maximum diameter was measured by Vernier Callipers (d) FNAC findings (e)Fresh Vs Retreatment cases (f) ESR in mm 1st hour (g) prescription of DOTS Vs Non DOTS therapy (h) Occurrence of Paradoxical Upgrading Reaction (PUR) during the course of treatment. The last parameter was obviously added during the course of treatment. A total of 94 patients who could be followed up till the end of treatment were included in final statistical analysis.

FNAC findings were further categorized into four subgroups (A) Epitheloid granulomas without necrosis (B) Epitheloid granulomas with necrosis (C) Necrosis with granulomas with neutrophilic infiltrates.(D) Numerous macrophages only.[4] Subcategory A and D were labelled as tuberculosis only after clinical improvement with ATT.

As per INDEX TB guidelines, patients with a single node > 1cm in maximum diameter at the end of stipulated treatment duration were categorized as having persistent lymphadenopathy/partial responders.[6] Comparative analysis was done with patients whose lymph node(s) had resolved completely at the end of prescribed treatment with respect to all the hypothetical factors mentioned above. Also, extended treatment of 3–6 months in continuation phase was given and results were then compared. No steroids were given during the occurrence of PURs, rather only pain killers with Non Steroidal Anti Inflammatory drugs (NSAIDS) were administered.

2.1. Exclusion Criteria

(a) HIV positive patient *(b)* Patients with Diabetes Mellitus *(c)* Patients diagnosed as "Rifampicin Resistant" on PCR based assay *(d)* Patients with concomitant Pulmonary Tuberculosis based on Chest radiograph and/or sputum examination. *(e)* Patients with apparent/obvious immunosuppression e.g, on steroids.

2.2. Outcome

A lymph node >1.0 cm in residual size at the end of treatment (6 or 8 months) was considered as persisting lymphadenopathy/partial responder.[5] This was classified as partial-responders and the rest as responders.

3. Statistical analysis

We have used Epi-Info 7 software for analysis. Categorical variables are summarized as count and proportion while numerical variables as mean and standard deviation. Difference in distribution of various clinic–epidemiologic parameters across non-resolution and resolution group was compared by using Chi-square test or Mann–Whitney test appropriately. Unadjusted odds ratios and their confidence intervals are presented. To identify independent predictors of non-resolution we have used binary logistic regression analysis. Independent variable selection was done by data driven (variables with $p < 0.25$ on bivariate analysis) approach and also clinically plausible variables were selected. Hosmer–Lemeshow Goodness of Fit and Omnibus Test were used to evaluate fitness and significance. Adjusted odds ratios with their 95% confidence interval are presented.

4. Results

Patients were followed up till the end of prescribed treatment which consisted of 94 patients. Out of these 94 patients, 60 patients had complete resolution of lymph node(s) [R group] wheras 34 patients had residual lymphadenopathy.[PR group]. Both the groups were age matched with the mean age being 26.85 ± 12.10 and 27.35 ± 13.16 in PR and R group respectively. The number of males were equal i.e. 17 in each group but females were much more in number in the R group (43 Vs 17). However, this differential distribution of gender was not statistically significant in multivariate analysis (p value:0.522). The R group had significantly more proportion of solitary lymph node at presentation (73.02%) as compared to PR group (26.98%). This difference was significant with a p value of 0.016. The site of lymphadenopathy i.e. cervical Vs non cervical was not a significant variable but presence of multiple lymph nodes ($\geq$2 in number) and average size of lymphadenopathy at the time of presentation were statistically

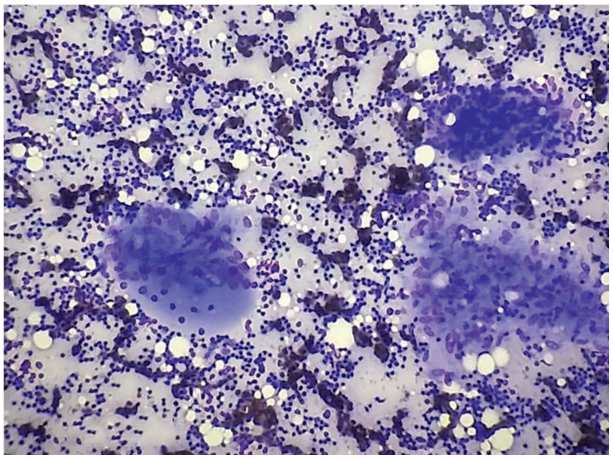

Fig. 1 – The photomicrograph shows epithelioid cell granulomas without necrosis (Pattern A) (Wright Giemsa; X100).

significant predictors of non resolution of lymphadenopathy (p value: 0.01 and 0.025 respectively). FNAC findings were categorized into four subgroups as described above (Figs. 1–4). It was found that presence of necrosis (subgroups B: Fig. 2 and C: Fig. 3) was significantly associated with non resolution/ partial resolution of lymph nodes as compared to subgroups A (Fig. 1) and D (Fig. 4) where necrosis was absent.78.8% of patients with A/D FNAC findings had resolved their lymph nodes at treatment completion whereas only 56.8% of B/C FNAC findings (presence of necrosis) had their nodes resolved at the end of prescribed treatment. Also, the proportion of PR cases was statistically indifferent in fresh (Category I) Vs retreatment cases (Category II)and also in intermittent Vs daily therapy. PURs were found to have a significant corelation with non resolution/partial resolution of lymph nodes at the end of treatment. PURs were noted in only 21.43% cases in the R group as compared to 78.57% in the PR group. Continuation phase was extended by 3–6 months to the 34 patients categorized as partial responders. Lymph nodes regressed in 26 out of these 34 patients at the end of 12 months leaving only 8 patients out of total 94 with persisting nodes at the end of one year.

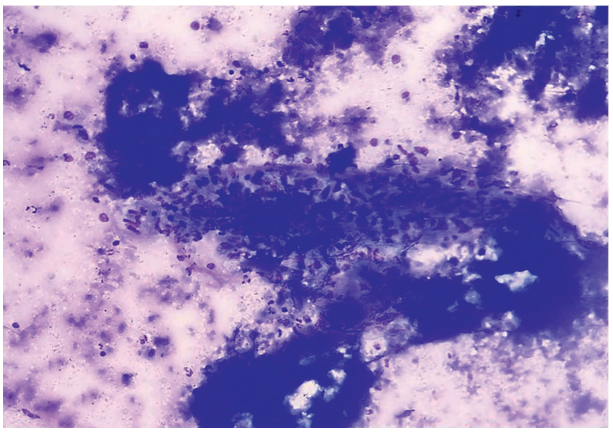

Fig. 2 – The photomicrograph shows epithelioid cell granulomas with necrosis (Pattern B) (Wright Giemsa; X200).

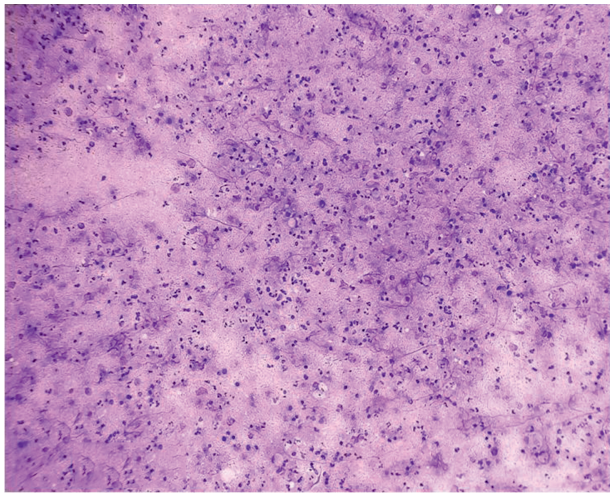

Fig. 3 – The photomicrograph shows dense mixed inflammation comprised predominantly of neutrophils in a necrotic background (Pattern C) (Wright Giemsa; X200).

5. Discussion

5.1. *Epidemiology*

Tubercular lymphadenitis usually affects patients in their second decade but can occur in any age group. Mean age in our cohort was 27.15 years with no difference in both the comparative groups. Female predilection of approximately 2:1 has been reported in western literature.[2,7] 60 out of 94 patients in this study were females (1:56:1). Also, increased frequency of tubercular lymphadenitis has been reported in Asian population.[8,9] Though the proportion of females in the PR group was higher but the inference was insignificant in multivariate analysis.

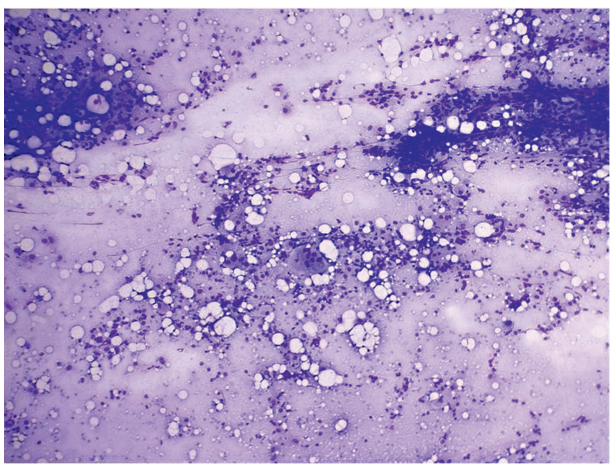

Fig. 4 – The photomicrograph shows numerous foamy macrophages and occasional histiocytic giant cell neutrophils (Pattern D) (Wright Giemsa; X100).

5.2. *Site and extent of involvement*

Cervical lymph nodes are the most common site of involvement and the same was true of our cohort with almost 90% presenting with cervical involvement (81/94). The site of involvement did not corelate with the treatment response as shown in Table 1. Also, a subjective assessment was done by counting the number of peripheral lymph nodes and calculating the average size of lymphadenopathy. A higher average size of lymph node at the time of presentation as well as presentation with multiple lymph nodes was significantly associated with the partial resolution of lymphadenopathy. Presence of discharging sinus or abcess was hardly encountered at the time of presentation and were not enrolled in the study.[10] We did not come across any such detailed data analysis in the literature. Although this parameter had limitations that absence of USG guided measurement and lack of concomitant CECT thorax did not give a more accurate extent of (mediastinal) lymphadenopathy yet the authors are of the opinion that these could be used as an early predictor of partial responders in patients of tubercular lymphadenitis.

5.3. *Paradoxical upgrading reaction*

After the patients of tuberculous lymphadenitis are put on ATT, they may suffer from enlargement of existing nodes or occurrence of new lymph nodes. Also labelled as paradoxical upgrading reaction, this phenomenon represents a kind of immune reconstitution, mostly seen during the intensive phase of treatment. This occurs in 10–30% of patients of tuberculous lymphadenopathy.[11,12] In our study, PUR occurred in 21.43% patients of the R group as compared to 78.57% in the PR group. The difference was highly significant with a p value of <0.001. Hence, occurrence of PUR in the intensive phase could be read as a strong predictor of persistence of lymph nodes at the end of prescribed treatment. It needs to be emphasized here that a variety of treatment options have been used to combat PURs which include surgical excision, corticosteroids, NSAIDS and also monoclonal antibodies.[13,14] Since there are no definitive guidelines for management of such patients, we used only NSAIDS for symptomatic relief and steroids/surgical excision was not done in any patient. Spontaneous resolution with just continuation of routine ATT occurs in majority of cases. Antigravity aspiration was done in 8 patients in which abscess formation occurred. The authors would emphasize here that none of the patients had any persisting sinus formation at the end of the treatment.

5.4. *FNAC findings*

FNAC is a rapid, safe and inexpensive tool in diagnosis of lymph node lesions.[15] For TB lymphadenitis, the technique was first used way back in 1927.[16] In the current study, FNAC findings were categorized into four subgroups (A) Epitheloid granulomas without necrosis (28 patients: 29.74%) (B) Epitheloid granulomas with necrosis (31 patients: 32.97%) (C) Necrosis with granulomas with neutrophilic infiltrates (30 patients: 31.91%).(D) Numerous macrophages only (5 patients: 5.31%). Presence of AFB positivity was 8/28 patients in subgroup A, 17/31 in subgroup B,19/30 in subgroup C and 0/5 in subgroup D. This is in concordance with existing literature that AFB positivity was higher in patients with necrosis on cytomorphology.[17] As evident in Table 1, 78% of patients with findings A/D on FNAC (No necrosis) had their nodes resolved at the end of treatment whereas only 56% patients with FNAC findings B/C (necrosis present) had resolution of respective lymph nodes at the end of prescribed treatment. It needs

Table 1 – Statistical corelation of proposed determinants with resolution of tubercular lymphadenopathy.

Determinant	Lymph Nodes Not Resolved (NR:34)	Lymph Nodes Resolved (R:60)	Univariate Analysis		Multivariate Analysis	
			OR (95% CI)	p-value	OR (95% CI)	p-value
Mean Age	26.85 ± 12.10	27.35 ± 13.16	0.99 (0.96–1.03)	0.856	–	–
Gender						
• Male	17 (50%)	17 (50%)	2.52 (1.05–6.07)	0.03	1.99 (0.53–7.47)	0.306
• Female	17 (28.33%)	43 (71.67%)				
Number of Nodes						
• Multiple	17 (54.84%)	14 (45.16%)	3.28 (1.33–8.09)	0.008	6.87 (1.41–33.3)	0.016
• Single	17 (26.98%)	46 (73.02%)				
Average size of lymph node (cm)	2.37 ± 1.3	1.88 ± 0.88	1.52 (1.01–2.30)	0.115	2.06 (1.09–3.9)	0.025
Site						
• Cervical	30 (37.04%)	51 (62.96%)	1.32 (0.37–4.67)	0.662	–	–
• Non cervical	4 (30.77%)	9 (69.23%)				
FNAC findings						
• C/B	27 (44.2%)	34 (56.8%)	2.94 (1.1–7.82)	0.026	7.60 (1.29–44.63)	0.024
• A/D	7 (21.2%)	26 (78.8%)				
Category						
• II	7 (35%)	13 (65%)	0.93 (0.33–2.63)	0.902	–	–
• I	27 (36.49%)	47 (63.5%)				
PUR						
• Present	22 (78.57%)	6 (21.43%)	16.5 (5.5–49.48)	<0.001	49.06 (8.93–269.2)	<0.001
• Absent	12 (18.18%)	54 (81.82%)				

10,000–100,000 AFB/ml of sample to be smear positive.[18] Presence of necrosis and/or AFB smear positivity on FNAC may be taken as a surrogate marker for higher bacillary load and hence may be the possible explanation of partial resolution at the end of prescribed ATT.

5.5. *Daily Vs intermittent therapy*

A few patients (n = 12) were on daily treatment (Non-DOTS) for different logistic reasons. As it is apparent that the resolution of lymph nodes did not differ whether the patients were on intermittent or daily therapy. It is to be noted that the number in daily treatment group was very less as all guidelines suggested intermittent therapy (Daily DOTS was not implemented when this study was carried out). Equal efficacy of daily and intermittent therapy in tuberculosis management has been described beforehand in literature for both pulmonary tuberculosis and tubercular lymphadenitis.[19,20]

5.6. *Limitations of the study*

There are a few limitations in the study which we would like to mention here,(i) Mycobacterium cultures of lymph node aspirates were not done in all patients as a goldstandard of diagnosis and drug sensitivity testing. However, Genexpert assay was done in all patients which ruled out practically all MDR-TB patients initially at the time of enrollment. Mycobacterium culture was also required in the 8 patients with persisting nodes at the end of 12 months which could have picked up Non-tuberculous mycobacteria, if any.(ii) Patients were labelled as having isolated peripheral lymphadenopathy based on chest radiograph only. Chest CT scans were not done because of ethical issues and concomitant mediastinal lymphadenopathy was hence not ruled out in all patients.(iii) Peripheral lymph nodes were measured only with the help of Vernier Callipers. Ultrasonographic assessment of lymph nodes could have more accurately described size and status of lymph nodes (iv) Because the treatment of PUR during ATT treatment is controversial, we stuck to only NSAIDs for symptomatic relief. Treatment with steroids may have led to a different result. Also, we did not offer surgical en bloc excision of lymph nodes, especially those with solitary ones since this has not been a proven therapeutic modality offered in literature.

6. Conclusions

Despite the limitations mentioned above, the results of this study not only provide us with certain predictors of partial responders before treatment but further raise more questions on the differential course of illness and hidden facts in the pathogenesis of the disease process. Occurrence of PURs, presence of bilateral and multiple lymph nodes and particular FNAC findings can alert the treating physician about possible poorer outcome in this subset of patients. Being patient and extending the continuation phase by 3–6 months may help overcome the problem of persisting lymph nodes in majority of patients. However, universal availability of mycobacterial culture and drug sensitivity testing to at least first line ATT drugs is the need of the hour as per standards of TB care. Another attempt of a similar study with larger number of patients and overcoming the listed limitations would be more useful to the policy makers to address the controversial issues in management of TB lymphadenopathy.

Authorship statement

The manuscript has been approved by all authors. All the authors have played important role in the diagnosis as well as contributed to the manuscript.

Conflicts of interest

The authors have none to declare.

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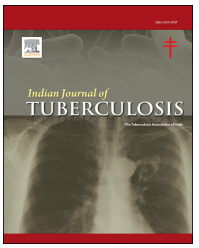

Original Article

Interferon-γ (+874 T/A) and interleukin-10 (−1082 G/A) genes polymorphisms are associated with active tuberculosis in the Algerian population of Oran's city

Mohamed Ghermi [a,d,g,*], *Sofiane Reguieg* [b], *Khadidja Attab* [a,d], *Nedjma Mened* [c], *Naima Ghomari* [c], *Fatima Zohra Guendouz Elghoul* [c], *Fatma Saichi* [c], *Saliha Bossi* [c], *Youcef Bouali-Youcef* [b], *Mohammed Bey Baba Hamed* [d,e], *Maryam Kallel Sellami* [f,g]

[a] *Biotechnology Department, University of Oran1 Ahmed Ben Bella, Oran, Algeria*
[b] *Immunology Department, EHU 1er Novembre Hospital, Oran, Algeria*
[c] *Tuberculosis and Lung Disease Control Service (UCTMR) of Essenia, Oran, Algeria*
[d] *AQUABIOR Laboratory, University of Oran1 Ahmed Ben Bella, Oran, Algeria*
[e] *Higher School of Biological Sciences of Oran (ESSBO), Oran, Algeria*
[f] *Immunology Department, La Rabta Hospital, Tunis, Tunisia*
[g] *Laboratory of Immuno-Rheumatology (LR05 SP01), La Rabta Hospital, Tunis, Tunisia*

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ABSTRACT

Background and aims: Polymorphisms within genes encoding the cytokines involved in anti-tuberculosis immunity have been widely studied and sometimes associated with an increased risk of developing the active form of tuberculosis (TB). This study analyzes for the first time the impact of two polymorphisms, namely *IFNG*+874 T/A and *IL10*-1082 G/A, in the Algerian population where tuberculosis is moderately endemic.

Methods: This case–control study included 104 healthy controls and 141 active TB patients: 75 extrapulmonary (EPTB) and 66 pulmonary (PTB). They were all genotyped by refractory mutational system-PCR amplification. In order to measure the functional impact of *IFNG*+874 T/A on the production rate of IFN-γ, 43 patients performed a QuantiFERON®Gold In-tube test.

Results: The *IFNG*+874 AA genotype was associated with a higher risk of developing EPTB (OR = 2.52; 95%CI = 1.23–5.18; $p = 0.012$) while the *IFNG*+874 TA genotype was associated with a greater protection (OR = 0.34, 95%CI = 0.16–0.74; $p = 0.006$) which was further characterized by a high production of IFN-γ ($p = 0.001$). Similarly, the allele A of SNP *IL10*-1082 G/A, especially in its homozygous form (AA), were overrepresented in PTB patients ($p = 0.010$ and 0.019, respectively). The combination of both susceptibility genotypes (AA/AA) was strongly associated with risk of development of active TB (OR = 8.58; 95% C.I = 1.95–37.70, $p = 0.004$). This susceptibility combination was only significant in men regarding

* *Corresponding author*. Biotechnology Department, Faculty of Sciences of Nature and life, University of Oran1 Ahmed Benbella, B.P. 1524 El Mnaouar, Oran, 31000, Algeria, Tel.: +213666893391.
E-mail address: ghermi.mohamed@univ-oran1.dz (M. Ghermi).
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PTB (OR = 11.05; 95% C.I = 1.32–92.72, $p = 0.027$). Additionally, *IFNG*+874 TA and *IL10*-1082G* genotypes combination was mostly encountered in men controls and conferred the highest protection rate against EPTB (OR = 0.25; 95% C.I = 0.08–0.76, $p = 0.015$).

Conclusion: These two cytokines genes polymorphisms are associated with active TB susceptibility in the Algerian population. They act synergistically in terms of protection and susceptibility regarding the two forms of the disease. Moreover, these associations were more marked among males suggesting a potential role of gender.

1. Introduction

According to the World Health Organization, nearly 20 persons develop active tuberculosis (TB) every minute and two patients die from it during the same time.[1] This disease, mainly caused by *Mycobacterium tuberculosis* (*Mtb*), cannot be explored by the unique microbiological paradigm as said by G. Bacelli in 1882: « Il bacillo non é ancora tutta la tuberculosi » [The bacillus is not yet all tuberculosis].[2] Historically, before R. Koch discovered[3] the causative agent of tuberculosis, the genetic component had long been suspected through numerous observations.[4] While in the majority of cases the initial multiplication of the pathogen is controlled by the effectors of the host's immune system, only 5–10% of the infected individuals develop the active form of the disease.[5] This unequal susceptibility facing TB infection is explained in part by the immunogenetic background of individuals since the production of immune effectors (cytokines, chemokines, receptors, co-stimulation molecules, etc.) is modulated by numerous functional single nucleotide polymorphisms (SNPs).[6] The cooperation of the alveolar macrophages, which engulf Mtb, with the specific CD4+ T lymphocytes is the key element that allows an efficient anti-tuberculosis immunity. This latter is characterized by the formation of the granuloma where culminates all the cellular effectors.[7] Interferon-gamma (IFN-γ) and Tumor necrosis factor-alpha (TNF-α) are the two main pro-inflammatory cytokines which act synergistically on the infected macrophage. Their combined effects overcome the blockade of phagolysosomal maturation, activate bactericidal mechanisms (reactive oxygen and nitrogen species), increase antigen presentation and stimulate the production of several cytokines and chemokines.[8] Interleukin-10 (IL-10) is an anti-inflammatory cytokine produced mainly by regulatory T-lymphocytes and certain macrophages to counterbalance the pro-inflammatory factors, which in case of excess can cause lung tissue lesions.[9] An effective immune response is characterized by a balance between pro-inflammatory and anti-inflammatory factors.[10] Many studies have explored, throughout association studies, the link between many SNPs in IFN-γ and IL-10 genes and the disease susceptibility in a multitude of populations from different ethnicities and led to very discordant outcomes.[11–13] To our best knowledge, this study is the first one exploring the impact of two polymorphisms, namely *IFNG*+874 T/A (rs2430561) and *IL10*-1082 G/A (rs1800896), and their combination on susceptibility to active tuberculosis (pulmonary and extrapulmonary) in the Algerian population.

2. Populations and methods

2.1. Study populations

This case–control study enrolled 245 Algerian subjects which are inhabitants of the Oran city. All the enrolled subjects have already given an informed written consent for participation in the study and the use of their personal and medical data. This study was conducted according to the declaration of Helsinki Principles and approved by the local scientific and ethics committee. The 141 patients (71 males) were ≥15 years and had a mean age (±SD) of 37.7 ± 14.7. They were recruited from December 2016 to July 2019 at the Tuberculosis and Lung Disease Control Service of Essenia (n = 100) and the Immunology department of EHU (1er Novembre 1954) hospital (n = 41), both located in Oran (Algeria).

The diagnosis and definition of pulmonary (PTB, n = 66) and extra-pulmonary (EPTB, n = 75) tuberculosis cases were made on the basis of the national anti-tuberculosis program manual which is inspired by the WHO guideline.[14,15] In addition to epidemiological and clinical context, imaging and microscopy were used to confirm PTB cases, and for EPTB cases: imaging, histology (necrotic caseofollicular granuloma) and/or a positive immunodiagnostic (tuberculin skin test ≥ 10mm or QuantiFERON® Gold In-tube) (Table 1). HIV serological status was available for 56 patients in whom it was negative.

The healthy control (HC) group consisted of 104 (59 males) consenting healthy blood donors who were enrolled at the blood transfusion center of CHU Oran in the same city (Oran). These individuals, without any familial link with the studied patients, had a mean age of (35.0 ± 11.5). They were all negative for HIV, HCV, HBV and syphilis serological diagnosis.

2.2. Molecular methods

Blood samples were collected on anticoagulant tubes (EDTA) and DNA was extracted by a conventional 'salting out' method following a protocol adapted from that of Lahiri et al.[16] After extraction, DNA was quantified, and quality was assessed by a spectrophotometric method (Nanodrop®). The two studied polymorphisms (*IFNG*+874 T/A and *IL10*-1082 G/A) were genotyped using an amplification refractory mutation system-PCR (ARMS-PCR) as previously described.[17,18] The sequences of all used primers and genotyping product sizes are listed in (Suppl.1). PCR program for *IFNG*+874 T/A genotyping was as follows: After 5 minutes of initial denaturation (95 °C), 13 cycles (15 seconds at 95 °C, 30 seconds at **63 °C**, and 40 seconds at

Table 1 – Criteria used for diagnosing TB cases.

TB type (n)	Microscopy (+)	Histology	Imaging	Immunodiagnostic[a]	Response to treatment
PTB (66)	59	1	6[b]	0	0
EPTB (75)	0	41	23[c]	7	5

[a] TST (≥10mm) and/or QFT-GIT (+).
[b] 3 miliary cases.
[c] 20 cases had a positive immunodiagnostic.

72 °C) followed by 17 cycles (15 seconds at 95 °C, 30 seconds at **57 °C**, and 40 seconds at 72 °C), and finally 5 minutes of final elongation at 72 °C. For *IL10*-1082 G/A, the same program was used with a slight modification in hybridization temperatures (65 °C and 58 °C). Amplified products (*IFNG*+874 T/A: 261 pb and *IL10*-1082 G/A: 550 pb) were monitored by electrophoresis on a 1% agarose gel containing ethidium bromide and thereafter visualized under ultraviolet light.

2.3. Interferon-gamma measurement

In order to analyze the impact of *IFNG*+874 T/A polymorphism on IFN-γ production, 41 patients had performed a Quanti-FERON® Gold In-Tube (QFT) test. It consists in quantifying, by an ELISA method, the T-cell IFN-γ production (ex-vivo) after stimulation with specific *M. tuberculosis* antigens. The tests were done according to the manufacturer's recommendations (Qiagen® Cellestis, Victoria, Australia). IFN-γ concentrations (IU/ml) without stimulation (serum level) and after antigen stimulation were measured and compared regarding the different polymorphisms' genotypes.

2.4. Statistical analysis

All data were recorded and analyzed using the IBM SPSS Statistics 21.0 software. A *p*-value less than 0.05 has been set for statistical significance. The Hardy–Weinberg equilibrium (HWE) was checked in both groups. A comparison between quantitative and qualitative variables was carried out by a student (*t*-test) when normally distributed; otherwise, non-parametric Mann–Whitney and Kruskal–Wallis tests were used. Measured IFN-γ concentrations (IU/ml) were expressed as median (interquartile range, IQR). Allelic and genotypic frequencies were compared using Chi-squared or Fisher's exact test. In addition to the additive and multiplicative model, we analyzed recessive, dominant and heterozygous genetic models of association. Binary logistic regression was used to calculate the Odds Ratios (OR) with 95% confidence interval (95%C.I) after adjustment for sex and age.

3. Results

3.1. Patients' characteristics

The mean age of PTB (37.0 ± 14.3) and EPTB patients (38.4 ± 15.0) was very similar. However, a strong significant difference ($p < 0.000$) was observed in terms of sex distribution between these two localizations. While individuals with PTB were predominantly males (72.7%), women represented the majority of EPTB cases (69.3%).

Among PTB patients, 89.4% were positive for acid-fast bacilli microscopy and the most frequent lesions profile was characterized by a mild to severe extent (96.5%) and presence of cavitation (58,6%). Lymph node localization was at the main EPTB form (40.0%) followed by pleural (17.3%) and abdominal involvement (12.0%).

3.2. Polymorphisms analysis

The analysis of genotype frequencies distribution showed that they were, for *IL10*-1082 G/A, consistent with Hardy Weinberg equilibrium in controls ($\chi 2 = 0.001$; $p = 0.973$) and patients ($\chi 2 = 0.299$; $p = 0.584$). For *IFNG*+874 T/A, equilibrium was found only in patients ($\chi 2 = 0.555$; $p = 0.456$). All allelic and genotypic frequencies of the two studied polymorphisms are mentioned in (Table .2).

3.2.1. Association of IFN-γ gene polymorphism to active tuberculosis susceptibility

Genotype frequencies analysis of *IFNG*+874 T/A polymorphism showed a significant difference between controls and TB patients ($p = 0.018$). After adjusting for age and sex, the AA genotype were found to be a two-fold more frequent in TB patients (OR = 2.07; 95%C.I = 1.13–3.79, $p = 0.019$). Conversely, a significant negative association was clearly evident between the heterozygous genotype (TA) and TB group compared with controls suggesting its protection role (OR = 0.43; 95% C.I = 0.23–0.81, $p = 0.009$).

Regarding TB localization, these associations were restricted to EPTB patients: genotypic ($p = 0.014$), AA susceptibility genotype (OR = 2.52; 95%C.I = 1.23–5.18, $p = 0.012$), and TA protection genotype (OR = 0.34; 95%C.I = 0.16–0.74, $p = 0.006$). These findings suggest that it is inappropriate to combine PTB and EPTB tuberculosis patients when performing such association's studies.

After stratification according to gender (Suppl.1), we found that this polymorphism was differently distributed. In fact, AA genotype was more frequently associated to extra-pulmonary tuberculosis when comparing between patients and controls of male sex (OR = 3.05; 95%C.I = 1.07–8.65, $p = 0.036$). Additionally, the protection conferred by TA genotype was circumscribed to the EPTB localization in men (OR = 0.22; 95% C.I = 0.07–0.71, $p = 0.011$).

3.2.2. Association of IL-10 gene polymorphism to active tuberculosis susceptibility

Analysis of the distribution of the *IL10*-1082 G/A polymorphism revealed a very significant difference in terms of allelic frequencies ($p = 0.002$) with a predominance of A allele in patients (OR = 1.75; 95%C.I = 1.22–2.52) and of the G allele in HC (OR = 0.57; 95%C.I = 0.40–0.82). The double carriers of the

Table 2 – Allelic and genotypic frequencies of IL10-1082 G/A and IFNG+874 T/A polymorphisms.

		HC (104)	TB (141)	PTB (66)	EPTB (75)
IL10 -1082 G/A	**A (%)**	47.1	61.0	61.4	60.7
	p		**0.002**	**0.010**[a]	**0.011**[b]
	OR (95% CI)		**1.75 [1.22–2.52]**	**1.78 [1.14–2.78]**	**1.73 [1.13–2.65]**
	GG (%)	29 (27.9)	23 (16.3)	10 (15.2)	13 (17.3)
	GA (%)	52 (50.0)	64 (45.4)	31 (47.0)	33 (44.0)
	AA (%)	23 (22.1)	54 (38.3)	25 (37.9)	29 (38.7)
	p		**0.011**	**0.039**[a]	**0.039**
IFNG +874 T/A	**A (%)**	51.0	56.4	55.3	57.3
	p		NS	NS	NS
	OR (95% CI)				
	TT (%)	18 (17.3)	29 (20.6)	13 (19.7)	16 (21.3)
	TA (%)	66 (63.5)	65 (46.1)	33 (50.0)	32 (42.7)
	AA (%)	20 (19.2)	47 (33.3)	20 (30.3)	27 (36.0)
	p		**0.018**	NS	**0.014**[a]

NS: Not signification ($p > 0.05$).
[a] Significant association ($p < 0.05$) in males.
[b] Significant association ($p < 0.05$) in females.

susceptibility allele AA were overrepresented among TB patients as compared with HC subjects (OR = 2.23; 95% C.I = 1.25–4.01, $p = 0.007$). Inversely, GG genotype was markedly more associated to protection regarding active TB (OR = 0.50; 95% C.I = 0.27–0.93, $p = 0.029$).

These genotypes were associated to increased susceptibility (AA) and protection (GG) against the two forms of the active disease (pulmonary and Extrapulmonary) when analyzed separately (Tables 2 and 3). Nonetheless, these associations were observed only in males for PTB (AA: OR = 2.77; 95%C.I = 1.13–6.80, $p = 0.026$ and GG: OR = 0.29; 95% C.I = 0.10–0.85, $p = 0.024$), and in females for EPTB (AA: OR = 2.51; 95%C.I = 1.05–6.03, $p = 0.039$) (Suppl.1).

3.2.3. *Combination of* IFNG+874 T/A *et* IL10-1082 G/A

The nine possible combinations of genotypes of both polymorphisms were found in TB and HC groups (Table .4) where their frequencies were significantly different ($p = 0.013$). Although the *IFNG*+874 AA genotype could not be associated with PTB when it was analyzed alone, its combination to the *IL10*-1082 AA genotype was very strongly associated with an increased risk of PTB occurrence (OR = 9.82; 95% C.I = 2.08–46.38, $p = 0.004$). Moreover, the combination (TA/G*) was found to be the most protective one, in particular vis-à-vis EPTB with a 0.35-fold risk as compared with other genotype combinations ($p = 0.003$).

3.3. *Impact on the interferon-gamma production*

The overall median IFN-γ serum level (unstimulated) in patients who performed a QFT test (n = 43) was 0.18 IU/ml (IQR: 0.04–0.38). The median specific IFN-γ response to the anti-mycobacterial antigens (stimulated) was 1.20 IU/ml (IQR: 0.42–5.81).

Stimulated and unstimulated production levels of IFN-γ in terms of *IFNG*+874T/A genotypes are represented in (Fig. 1).

Table 3 – Analysis of the different genetic models of studied polymorphisms.

SNP	Genetic model	*p* – value		
		TB	PTB	EPTB
***IL10*-1082 G/A**	Multiplicative	0.002	0.010[a]	0.011[b]
	Additive	0.011	0.039[a]	0.011
	Recessive (AA *vs* GA + GG)	0.007	0.019	NS[b]
	Dominant (AA + GA *vs* GG)	0.029[a]	NS[a]	*NS*
	Heterozygous (GA *vs* GG)	*NS*	*NS*	*NS*
	Homozygous (AA *vs* GG)	0.004	0.014[a]	0.046
***IFNG*+874 T/A**	Multiplicative	*NS*	*NS*	*NS*
	Additive	0.018	*NS*	0.014[a]
	Dominant (AA + TA *vs* TT)	*NS*	*NS*	*NS*
	Recessive (AA *vs* TA + TT)	0.019	*NS*	0.012[a]
	Heterozygous (TA *vs* AA)	0.009	*NS*	0.006[a]
	Homozygous (AA *vs* TT)	*NS*	*NS*	*NS*

Logistic regression was performed to calculate *p*-values (adjustment for age and sex) by comparison with healthy controls.
[a] Significant association ($p < 0.05$) in males.
[b] Significant association ($p < 0.05$) in females.

Table 4 – Frequencies of genotype combinations and their associations with active TB.

Combination	HC % (n = 104)	TB % (n = 141)	OR [95% CI] P_c (p^a)	PTB % (n = 66)	OR [95% CI] P_c (p^a)	EPTB % (n = 75)	OR [95% CI] P_c (p^a)
AA//AA	01.9	14.9	8.58 [1.95–37.70] 0.004 (0.001)[b]	16.7	9.82 [2.08–46.38] 0.004 (0.0004)[b]	13.3	5.83 [1.18–28.86] 0.031 (0.003)
AA//GA	11.5	14.2	-	10.6	-	17.3	-
AA//GG	05.8	04.3	-	03.0	-	05.3	-
TA//AA	14.4	17.0	-	15.2	-	18.7	-
TA/G*	49.0	29.1	0.44 [0.26–0.75] 0.002 (0.001)[b]	34.8	-	24.0	0.35 [0.18–0.70] 0.003 (0.001)[b]
TT//AA	05.8	06.4	-	06.1	-	06.7	-
TT/GA	07.7	11.3	-	10.6	-	12.0	-
TT/GG	03.8	02.8	-	03.0	-	02.7	-

P_c: p adjusted (age and sex).
-: Not signification ($p > 0.05$).
[a] p not adjusted.
[b] Significant association ($p < 0.05$) in males.

Although unstimulated rates were not statistically different ($p = 0.57$), individuals carrying the genotype that confers protection (TA) were the largest producers of antigen-specific stimulated IFN-γ ($p = 0.001$) with a median rate of 5.76 IU/ml (IQR: 1.68–9.22). Moreover, the most protective genotype combination (TA/G*) was associated with the highest level IFN-γ in response to specific anti-mycobacterial antigens ($p = 0.007$) with a median rate of 6.99 IU/ml (IQR: 3.02–10.00).

4. Discussion

According to the latest WHO report (2019), tuberculosis is still a serious global health problem in Algeria which is considered to be a moderately endemic country given its annual incidence rate of about 70 cases/100.000 inhabitants.[19] Fortunately, only 5–10% of those infected will develop the active form of the disease while the majority of individuals effectively controls the mycobacteria and remains a carrier of latent infection.[20]

The first epidemiological studies have found that genetic factors contributed greatly to this inter-individual variability with an estimated heritability of 36–80%.[21,22] This finding was supported by the data from molecular studies in recent decades.[23] Studies examining the association of polymorphisms in genes encoding cytokines involved in the establishment of anti-tuberculosis immunity (e.g. IFN-γ, IL-10, TNF-α …) have produced very discordant results sometimes even within the same population.[6] The North African populations have been rarely and sometimes never for some cytokine gene

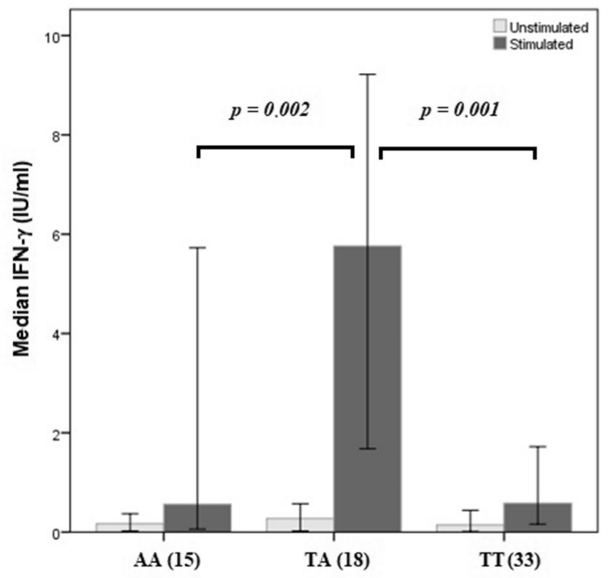

Fig. 1 – Impact of *IFNG*+874 T/A polymorphism on IFN-γ patients production levels. Median rate of IFN-γ production, without stimulation (serum level) and after antigenic stimulation in patients (n = 42) having performed a QFT. Error bars represent 95% confidence interval. Non parametric Mann–Whitney test was used to calculate *p*-values. Numbers in parentheses represent the number of individuals for each genotype.

polymorphisms. This study allows, for the first time, the exploration of the link between *IFNG*+874 T/A and *IL10*-1082 G/A polymorphisms, and the risk of occurrence of the two forms of active tuberculosis (pulmonary and extra-pulmonary) within the Algerian population.

The *IFNG*+874 T/A polymorphism (rs2430561) is one of the few SNPs identified within the IFN-γ gene (chromosome 12q14, intron1).[24] This is one of the rare polymorphisms having been associated with active form of tuberculosis in many populations.[12,13,25] We noticed through this study that the heterozygous *IFNG*+874TA genotype conferred protection against active tuberculosis in the Algerian population (OR = 0.43; $p = 0.009$). Conversely, the homozygous *IFNG*+874AA genotype was correlated with an increased risk of developing active tuberculosis, according to a recessive model (OR = 2.07; $p = 0.019$). These results are very similar to those observed by Ben Selma et al within the Tunisian population which is genetically close to our study population.[26] In fact, they observed that the two genotypes (TA and AA) were respectively associated with protection (OR = 0.46; $p = 0.0006$) and susceptibility to the disease (OR = 2.09; $p = 0.003$). Not only that, numerous meta-analyses have confirmed these observations. The most recent one, conducted by Wei et al,[25] involved 42 case–control studies comprising 8,574 patients and 9,011 controls. This meta-analysis found that the TA genotype was strongly associated with protection against active tuberculosis (OR = 0.71; $p = 10^{-6}$), and that the susceptibility genotype corresponded to AA (OR = 1.47; $p = 10^{-6}$). According to the same meta-analysis, it is very interesting to note that this polymorphism is particularly linked to tuberculosis susceptibility in populations belonging to Asian and African ethnic groups where it is nowadays the most endemic.

As the dynamics of the interactions between the immune system and the causative pathogen are different between the two main TB localizations,[27] it seemed to us more interesting to analyze distinctly the influence of these two polymorphisms. Indeed, the effects of protective (TA) and susceptibility (AA) genotypes were limited to the extra-pulmonary territory with higher risk ratios. This SNP, located at the 5′ end of a CA repetition, coincides with the NFκB transcription factor binding site (GGGAN'**T/A**'YYCC). The latter would have a stronger affinity for the T allele compared to the A allele, inducing a higher production of IFN-γ.[17,28] In our study, the analysis of production rates, after specific antigenic stimulation, found that carriers of the protective genotype were the largest producers ($p = 0.001$). This functional impact was also found in several studies with a higher production rate in T allele carriers.[18,29–31] Undoubtedly, IFN-γ is the major piece in the arsenal set up by the immune system initially to control the infection (formation of the granuloma) then to eliminate the pathogen (microbicidal effectors within macrophages).[32] Low levels of this cytokine induce undeniably the escape of the pathogen from the initial pulmonary focus to the extra-pulmonary territories. The Mendelian susceptibility to mycobacterial disease (MSMD) is the extreme consequence of failure of the IFN-γ–mediated immunity.[33] This syndrome, mainly characterized by severe and disseminated forms of mycobacterial infection, is due to mutations within one of the 15 genes involved in the IFN-γ/IL-12 axis which controls the cooperation between CD4+T cells and infected macrophages.[34]

Granulomas, which represent the seat of this cooperation between the various immune effectors, can generate cavitations that are associated with higher progression, transmission and treatment failure.[35,36] This alteration of the protective function of the granuloma is the consequence of a local inflammatory imbalance which causes their caseous necrosis and the shedding of their liquid content in the bronchi.[27] IL-10 is the main anti-inflammatory cytokine alongside TGF-β to ensure that pathogen eradication is achieved with minimum damage to the host.[37] Mice based-models had also confirmed the impact of the deleterious effect of IL-10 lack in terms of granuloma efficiency and histological distribution of immune effectors.[38] It has also been described that impaired production of this cytokine is associated with more lung dysfunctions and obstructive forms.[39] The association between the greatest risk of developing TB and excessive immune response is supported by observations made on cancer patients receiving immunotherapy. Indeed, an increasing number of patients on immune checkpoint inhibitors develop active TB.[40,41] Furthermore, some authors hypothesized that *Mtb* may drive an auto-immune/auto-inflammatory response to exacerbate inflammation through lipid antigens presentation.[10] It is then proposed that the development of TB reflects primarily a loss of tolerance.[42]

Like many cytokines, IL-10 production is modulated by certain gene polymorphisms. *IL10*-1082 G/A SNP (rs1800896), located in the promoter region (1q31), occurs within a putative Ets (E26 transformation-specific) transcription factor binding site.[11]

Through this study, it appears that (A) allele which is associated with decreasing IL-10 production[18] is a significant genetic marker of TB susceptibility within Algerian population following, rather, a recessive model. Homozygous (AA) carriers had a risk of more than 2 times greater than that of controls who had more frequently the other genotype (GG). This association was perceived for the two localizations of the infection, suggesting its important role in the genetic susceptibility to tuberculosis. In contrast to the first SNP analyzed, studies analyzing the role of the *IL10*-1082 G/A polymorphism regarding susceptibility to TB were very discordant.[11] The majority of these studies found no link (e.g. Indian, Pakistani, Spanish, Macedonian and Peruvian populations). For the few remaining studies, allele (A) represented the protective allele in the Tunisian and Egyptian populations, yet this allele was associated with an increased risk in the Gambian, Korean and Chinese populations as observed in our study population. These discordances could be attributed to the genetic heterogeneity between the studied populations that can be estimated through the high variability in minor allele frequencies observed in patients and controls in those studies.[11]

The absence of association, for the studied SNPs, within the Caucasian population suggests a strong selection pressure during the great epidemics' episodes which ravaged these European populations causing more than one billion deaths in the last 2000 years.[34]

Unlike MSMD, which appears to be monogenic, adult TB is much more complex. It involves several gene variants, hence the importance of analyzing combinations of SNPs from the candidate genes.[23,34] In fact, the two susceptibility genotypes (AA/AA) act in synergy leading to an almost 10 times higher risk concerning pulmonary localization. This phenotype may be characterized by a hyper-inflammatory profile (IL-10^{low}) combined with an inefficient granuloma structuration (IFN-γ^{low}). On the other side, the accumulation of the two protection genotypes (TA/G*) led to the greatest protection rate in particular regarding extra-pulmonary forms (OR = 0.35). The corresponding phenotype combines a high production of the most important anti-mycobacterial cytokine (IFN-γ^{high}) and a high production of interleukin 10 (IL-10^{high}) which rebalances the pro/anti-inflammatory equilibrium.

Of the 9 million adults who contracted TB in 2018, almost 65% were males.[1] This disparity between men and women has been noted for a very long time without finding the most exact explanation. Unequal access to health care has been studied and was found to be insufficient on its own to cause this disparity.[43] Many biological causes, notably hormonal, have been explored on the mouse model concluding for most of the studies on the immune-stimulatory role of estrogens contrary to the role of androgens which would be rather a factor of susceptibility.[44] Other authors proposed that there is a sex-specific genetic susceptibility given the high density of immune-related genes on the X chromosome.[45] Likely in our study, we observed that the two polymorphisms' effect (protection/susceptibility) were restricted mainly to the male sex as in the Tunisian population.[26] This may underlie an X-linked genetic mechanism as it was observed in some MSMD causal mutations. Additionally, two candidate regions (Xp11.4-Xp21.2 and Xq25-Xq26.3) were identified in those patients suffering from MSMD, some of them also had presented active tuberculosis.[46,47] The explanation of this sex-biased susceptibility can be provided by the exploration of the differences in immune gene regulation profile between males and females which is not limited to sex chromosomes.[48]

Discrepancies in term of genetic susceptibility between different studies can be also explained by the heterogenous nature of the Mtb-specific immune responses developed by infected individuals even if they have a similar immunogenetic background. Factors such as HIV co-infection and malnutrition or other acquired immunodeficiencies have a considerable impact on this response.[49] Moreover, the microbiota-epigenetics interlink is currently the subject of increased attention giving its involvement in gene expression modulation, which may explain the effect of environmental factors on the immune response.[50,51]

To conclude, this study demonstrated the importance of the stratification of patients according to the localization of the infection (pulmonary or not) when these association studies are carried out. We have also highlighted the utility to analyze polymorphisms' combination of genes involved in antituberculosis immunity. Thus, analyzing additional polymorphisms may give a more precise picture about the real implication of the genetics in the pathogeny of TB in the Algerian population. The role of the gender has also to be considered in future explorations of genetic susceptibility to the two forms of tuberculosis. Furthermore, including a group of latently infected individuals will undoubtedly help us to better understand the underpinning mechanisms of inter-individual variability regarding active tuberculosis. Therefore, it will improve preventive and therapeutic strategies in an attempt to eradicate this harmful infectious disease by focusing primarily on high risk populations.

Conflicts of interest

The authors have none to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijtb.2020.08.015.

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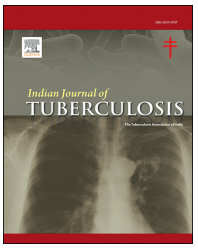

Original Article

FEV1/FEV6 is effective as a surrogate for FEV1/FVC in the diagnosis of chronic obstructive pulmonary disease

Palanisamy Venkatachalam, Dharm Prakash Dwivedi, Vishnukanth Govindraj*

Department of Pulmonary Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry, India

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ABSTRACT

Background and objective: Chronic Obstructive Pulmonary Disease (COPD) causes substantial morbidity and mortality across the globe. Diagnosis of COPD requires post-bronchodilator FEV1/FVC <0.70 as per GOLD Guidelines. FVC maneuver requires a minimum of 6 seconds of forceful expiration with no flow for 1 second for an accepted effort, which lacks any fixed cut-off point. This leads to discomfort, especially in advanced COPD and old aged population. We conducted this study to find the utility of FEV1/FEV6 as a surrogate for FEV1/FVC, the correlation between the two ratios, and the fixed cut-off value of FEV1/FEV6 for COPD diagnosis.

Methods: This was a prospective, cross-sectional study approved by the institutional ethics committee conducted from January 2017 to November 2018. Consented patients above 18 years suspected of COPD underwent Spirometry as per ATS guidelines. FEV1, FEV6, FEV1/FEV6 and FEV1/FVC ratios were recorded from the best acceptable maneuver.

Results: Out of 560 screened patients, 122 diagnosed as COPD. The correlation coefficient between the post-bronchodilator FEV1/FVC ratio and FEV1/FEV6 ratio was 0.972 ($p < 0.01$). The relationship between the post-bronchodilator FEV1/FVC ratio and FEV1/FEV6 ratio (linear regression analysis) was found out as: $FEV1/FVC = -1.845 + 1.009(FEV1/FEV6)$. Using this formula, the post-bronchodilator FEV1/FEV6 value of 71.845 was obtained corresponding to the post-bronchodilator FEV1/FVC value of 70.00.

Conclusion: We found a positive correlation coefficient ($r = 0.972$, $p < 0.001$) between the FEV1/FEV6 and FEV1/FVC ratios and the cut off value of 71.845 ($p < 0.01$) for the post-bronchodilator FEV1/FEV6 ratio for the diagnosis of COPD. Thus FEV1/FEV6 should be used as a surrogate for FEV1/FVC for the diagnosis of COPD.

* *Corresponding author*. Department of Pulmonary Medicine, JIPMER, Dhanvantari Nagar, Pondicherry, 605006, India.
E-mail address: dippyd@gmail.com (D.P. Dwivedi).
dpdwivedi

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1. Introduction

1.1. Background

Chronic Obstructive Pulmonary Disease (COPD) causes substantial mortality and morbidity worldwide. The estimated global prevalence of COPD was approximately 384 million (11.7%) in 2010.[1] An increasing number of smokers in developing nations and a rise in the number of the elderly population in developed economies is contributing to the increase in the incidence of COPD. Estimated deaths of 4.5 million are projected from COPD by 2030.[2,3] Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2018 criteria requires post-bronchodilator forced expiratory volume1/forced vital capacity (FEV1/FVC) < 0.70 to confirm the presence of persistent airflow limitation and hence the diagnosis of COPD.[4] FVC maneuver requires a patient to blow for at least 6 seconds (3 seconds for kids below 12 years) and it is accepted when there is no flow for 1 second or more. As COPD patients have dynamic compression of airways, they require more time to exhale (sometimes 12–20 seconds), which may cause exhaustion, dizziness, and syncope due to reduced venous return to heart.[5] There is no fixed cut-off for the duration of exhalation. FEV1/FVC fixed ratio of 0.7 also tends to overdiagnose COPD, especially in the elderly population. Various authors have proposed a lower limit of normal (LLN) as the cut-off criteria for the diagnosis of obstructive airway disease.[5–7] FEV6 (the volume of air forcefully exhaled in 6 seconds) has also been proposed as a replacement or surrogate for FVC in spirometry.[7–9] FEV6 maneuver has a distinct endpoint of six seconds and is less physically demanding than FVC. FEV6 is more reproducible and reliable than FVC for the diagnosis of COPD.[5] FEV6 can also be measured using portable handheld spirometers, which are inexpensive and easy to use.[8,9] This makes it suitable for mass screening and bedside use. Several studies have investigated the utility of FEV6 for FVC in the diagnosis of COPD and other obstructive pulmonary disorders.[10–12] Most of these studies in published literature are retrospective and have used different methods or measurements for FEV1 and FEV6.[5,9–15] Very few studies have been done in India regarding utilization of FEV6 instead of FVC.[16–19] Therefore, we conducted this study with the primary objective to find the utility of FEV1/FEV6 as a surrogate for FEV1/FVC in the diagnosis of COPD in the South Indian population. We also tried to find out the correlation between FEV1/FEV6 ratio and FEV1/FVC ratio to arrive at a fixed cut off value for FEV1/FEV6 ratio as compared to the fixed cut off value of FEV1/FVC in the diagnosis of COPD.

2. Methods

2.1. Study design & subjects

This prospective, cross-sectional study was conducted from January 2017 to November 2018 in a referral academic center for the treatment of pulmonary diseases in southern India. The institutional review board approved the study.

2.2. Inclusion criteria

Patients of 18 years or more presenting to the outpatient department with any of the symptoms suggestive of COPD like progressive and persistent shortness of breath worsening with exertion, chronic intermittent unproductive or productive cough were included into the study.

2.3. Exclusion criteria

Patients with obstructive lung disease other than COPD (e.g. asthma, bronchiectasis), pulmonary disease (e.g. tuberculosis, interstitial lung disease, lung cancer) were excluded from the study. Patients with contraindications for spirometry according to American Thoracic Society guidelines,[20] thoracic surgery in the past six months, acute respiratory infection in the last three weeks, uncontrolled cardiac disease in the last six months (e.g. unstable angina, congestive heart failure, arterial hypertension, arrhythmia), pregnant women were excluded from the study.

2.4. Study procedure

Patients meeting the inclusion and exclusion criteria underwent spirometry. Spirometry was done using Care Fusion Type Master ScreenTM PFT system (2012 make, CareFusion Germany 234 GmbH, Hoechberg, Germany), as per American Thoracic Society guidelines.[21] Both pre and post-bronchodilator values of these parameters were recorded. Post-bronchodilator spirometry was done after nebulizing the patient with salbutamol nebulization solution 2.5 ml (1 ml containing Salbutamol equivalent to 5 mg). COPD was diagnosed as per the GOLD criteria of post-bronchodilator FEV1/FVC <0.70. Three acceptable maneuvers were performed for each spirometry reading, and the spirometry measurement with the largest sum of FEV1 and FVC were chosen for analysis. FEV1, FEV6, FEV1/FEV6 ratio, and FEV1/FVC ratio were also recorded from the best maneuver. The spirometry tests thus obtained were analyzed for their quality and acceptability. The tests not reaching the 6-second expiration time were excluded from the study. The variables recorded from the patients were age, gender, symptoms with their duration, pack-years of smoking, biomass fuel exposure, and family history. The outcome measure recorded were FEV1, FEV6, FVC, pre, and post-bronchodilator FEV1/FEV6 ratio, FEV1/FVC ratio. The details of risk factors, like tobacco smoke, biomass fuel exposure, and family history of COPD were recorded. The calculation of pack-years was done as defined by the National Cancer Institute dictionary.[22] The pack years for patients with a history of bidi smoking were calculated as described by Pal H et al.[23] Bidi is a local cigarette made by rolling coarse tobacco in a dried Coromandel ebony or East Indian ebony leaf (local name temburni or tendu; botanical name: *Diospyros melanoxylon*). The patients were classified into four subgroups based on pulmonary involvement and GOLD guidelines[4]:

Stage 1: FEV1/FVC < 70% and FEV1 $\geq$ 80%.
Stage 2: FEV1/FVC < 70% and 50% $\leq$ FEV1 < 80%.
Stage 3: FEV1/FVC < 70% and 30% $\leq$ FEV1 < 50%.
Stage 4: FEV1/FVC < 70% and FEV1 < 30.

Table 1 – Patient demographic and spirometry characteristics.

PARAMETERS/GOLD STAGE	GOLD 1	GOLD 2	GOLD 3	GOLD 4	TOTAL
Subjects (n; %)	1 (0.8%)	50 (41%)	45 (36.9%)	26 (21.3%)	122 (100%)
Age (years; mean ± SD)	62	60.53 ± 10.2	60.53 ± 10.3	53.08 ± 10.05	57.59 ± 10.47
Male (%)	1 (0.8%)	49 (40.2%)	43 (35.2%)	24 (19.7%)	117 (95.90%)
Female (%)	0 (0%)	1 (0.8%)	2 (1.6%)	2 (1.6%)	5 (4.1%)
Duration of symptom (month; mean ± SD)	12	65.75 ± 49.22	68.8 ± 49.11	71.72 ± 37.56	69.35 ± 46.58
Dyspnoea (n; %)	1 (0.8%)	46 (37.7%)	42 (34.4%)	24 (19.7%)	113 (92.6%)
Cough (n; %)	1 (0.8%)	45 (36.9%)	34 (27.9%)	19 (15.6%)	101 (82.8%)
Wheeze (n; %)	0 (0%)	19 (15.6%)	22 (18%)	10 (8%)	51 (41.8%)
Never Smoker (n, %)	0 (0%)	11 (9%)	8 (6.5%)	2 (1.6%)	21 (17.2%)
Smoker (n, %)	1 (0.08%)	39 (31.9%)	37 (30.3%)	24 (18%)	101 (82.8%)
Pack-Years (years; mean ± SD)	9	8.85 ± 3.31	9.30 ± 4.09	7.66 ± 4.46	8.63 ± 3.49
Pack-Years (%)					
1–10	1 (0.8%)	31 (25.4%)	26 (21.3%)	19 (15.6%)	77 (63.1%)
11–20		8 (6.5%)	19 (15.6%)	5 (4.1%)	24 (17.2%)
Biomass fuel exposure (n, %)	1 (0.8%)	35 (28.7%)	35 (28.7%)	22 (18%)	93 (73.2%)
FEV1 (% Predicted; mean ± SD)	81	61.03 ± 8.15	39.47 ± 5.17	25.8 ± 2.39	46.02 ± 15.61
FEV1/FVC (%; mean ± SD) Pre	59.43	58.24 ± 6.63	54.32 ± 7.9	44.01 ± 5.56	53.15 ± 8.70
FEV1/FVC (%; mean ± SD) Post	65.49	58.53 ± 6.52	52.27 ± 7.4	44.48 ± 6.93	53.39 ± 8.73
FEV1/FEV6(%; mean ± SD) Pre	61.41	59.06 ± 6.85	53.29 ± 7	44.85 ± 5.06	54.24 ± 8.43
FEV1/FEV6(%; mean ± SD) Post	66	59.83 ± 6.53	53.39 ± 7.27	46.44 ± 6.6	54.77 ± 8.37

Abbreviations: COPD- Chronic Obstructive Pulmonary Disease, GOLD- Global Initiative for Chronic Obstructive Lung Disease, FVC- Forced Vital Capacity, FEV1- Forced Expiratory Volume in one second, FEV6- Forced Expiratory Volume in six seconds, SD-standard deviation, Post–Post bronchodilator value.
Table 1: shows general demographic and spirometry outcomes of the patients according to the GOLD staging of COPD.

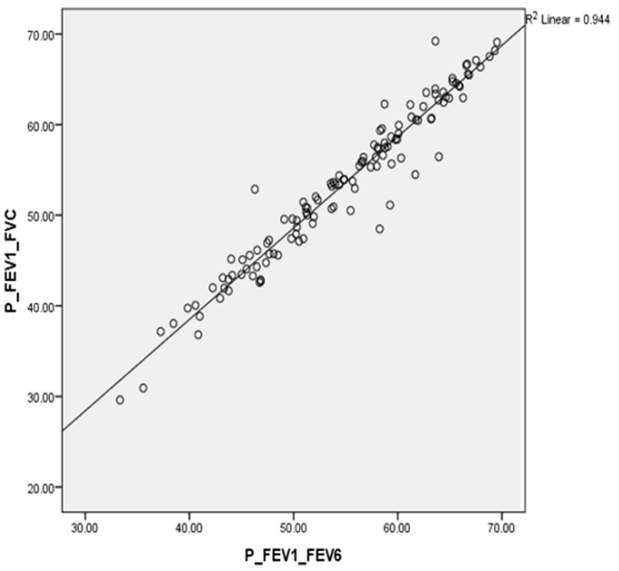

Fig. 1 – Linear Regression line between post-bronchodilator FEV1/FVC and postbronchodilator FEV1/FEV6 ratio showing R^2 value of 0.944; X axis: FEV1/FEV6, Y axis.

2.5. *Methods of statistical analysis*

Considering an expected sensitivity of 98% and specificity of 94% with the expected prevalence of COPD as 30% by spirometry (institute data), using the statistical formula for estimating a population proportion a sample size of 122 was calculated (95% confidence interval and 5% absolute precision). Statistical package for social sciences (IBM SPSS) version 19.0 was used for statistical analysis. The normality of the data was tested by a one-sample Kolmogorov–Smirnov test. The distribution of data for categorical variables and ordinal data such as gender, symptoms, presence of smoking, history of exposure to biomass fuel was expressed as percentages and frequencies. Continuous parametric data were expressed as mean and standard deviation. Continuous non-parametric data were expressed as median with range. Correlation between various variables was analyzed using Pearson's correlation analysis. Linear regression analysis was used to analyze the relationship between various variables such as FEV1/FVC ratio and FEV1/FEV6 ratio. All statistical tests were carried out at a 5% level of significance and p-value <0.05 was considered as statistically significant.

3. Results

Five hundred forty patients were suspected clinically for COPD and underwent spirometry test. One hundred and twenty-two patients satisfied GOLD diagnostic criteria of FEV1/FVC <0.7 and were diagnosed as COPD. There were 117 (95.9%) males and 5 females (4.1%) in the study. Most of the patients (91, 74.6%) were above 50 years of age. Ninety-three percent of patients (114/122) had 2 or more symptoms at presentation. The most common risk factor for COPD was smoking (n = 101, 82.8%) followed by Biomass fuel exposure (n = 93,73.2%). Most of the male patients (84/117, 71.8%) had exposure to both biomass fuel and smoking. The mean smoking history was 8.63 (SD ± 3.49) pack years. Almost two-thirds of them had a 1–10 pack-years history of smoking. Patients were classified based on their post-bronchodilator FEV1 values into various

Table 2 – Shows Cut-off values for FEV1/FEV6 found in various studies ([14–16,25,26,28,29,30]).

Study	Country	Year	Number of subjects	Correlation coefficient	Cut-off of FEV1/FEV6 ratio
Current study	India	2016–18	122	0.972	0.72
Frith et al (26)	Australia	2011	204	0.72	0.75
Ching et al (16)	Malaysia	2015–16	117	0.636	0.75
Rosa et al (28)	Brazil	2007	963	0.92	0.75
Wang et al (29)	China	2016	767	0.954	0.72
COPD Gene trial (14)	United States	2013	10,018	0.90	0.73
Singh and Lohia (25)	India	2008–09	467	0.93	0.73
Aghili et al (15)	Iran	2013	318	Not Studied	0.71
Melbye et al (30)	Norway	2006	3874	0.86	0.73

GOLD grades for the severity of obstruction. There were 50 (41%) and 71 (58.2%) patients in GOLD category 2 (moderate) and 3 & 4 (severe and very severe) respectively. The demographic and spirometry outcomes are presented in Table 1.

3.1. *Spirometry correlation*

There was a significant correlation ($r = 0.972$, $P < 0.01$) between post bronchodilation FEV1/FVC and FEV1/FEV6 ratios (Fig. 1). The relationship between the post-bronchodilator FEV1/FVC and FEV1/FEV6 ratios (linear regression analysis) was found to be FEV1/FVC = −1.845 + 1.009(FEV1/FEV6). Using this formula, the post-bronchodilator FEV1/FEV6 value of 71.845 ($p < 0.01$) was obtained corresponding to the post-bronchodilator FEV1/FVC value of 70.00 for the diagnosis of COPD.

We could not find any statically significant correlation between Post Bronchodilation FEV1/FVC and pack years smoking ($r = -0.136$, p-value 0.135) or duration of symptoms ($r = -0.078$, p-value 0.394).

Post Bronchodilator FEV1/FEV6 had both sensitivity and specificity of 100% when compared to Post bronchodilator FEV1/FVC of 0.70 for diagnosing COPD. The positive predictive value, as well as negative predictive value, were 100%.

4. Discussion

We found a positive correlation between post-bronchodilator FEV1/FVC and FEV1/FEV6 ratios in the present study which demonstrate that FEV1/FEV6 should be used as a surrogate for FEV1/FVC in the diagnosis of COPD. A positive correlation coefficient ranging from 0.636 to 1.0 for the FEV1/FVC and FEV1/FEV6 ratios has been reported by different investigators.[5,14,16,24–30] We obtained a post-bronchodilator FEV1/FEV6 value of 71.845 ($p < 0.01$) corresponding to the post-bronchodilator FEV1/FVC value of 70.00 for diagnosis of COPD. The findings of our study are like other published studies.[5,14,16,24–30] A comparative analysis of cut-off values and correlation is given in Table 2. In the COPD Gene trial by Bhatt et al, a Cohen's Kappa coefficient of 0.90 ($p < 0.001$) between the two ratios was found, indicating a good agreement.[14] The investigators reported that the FEV1/FEV6 cut-off value of 0.73 was significantly associated with COPD Quality of life, functional indices, and CT measures of emphysema and found it to be superior to FEV1/FVC in predicting future exacerbations and COPD related morbidity.[14] Enright and colleagues found that FEV1/FEV6 can be used to predict lung function decline over time.[31] They further stated that the shorter duration of the FEV6 maneuver was easy to perform and the FEV1/FEV6 ratio is a good substitute for the FEV1/FVC for the screening of smokers for the presence of airflow obstruction. A meta-analysis by Jing et al. concluded that FEV1/FEV6 is a sensitive and specific test for the diagnosis of airway obstruction and can be used as a valid alternative for the FEV1/FVC in the diagnosis of airway obstruction.[32]

Most of the studies done were retrospective in nature and analysed spirometry data of studies with different aims and objectives to start with.[5,10,13–18] While accessing the correlation between two ratios either two separate efforts or instruments or the different operators were used by several studies. Ching et al. used a small handheld device (COPD-6™) for FEV1/FEV6 measurement and office spirometry for the FVC maneuver and hence could not find a strong agreement between the two ratios ($r = 0.634$).[16] Similarly, Firth et al. used handheld expiratory flow meter (PiKo-6®, nSpire Health, Inc.) to assess the validity of the instrument and used FEV6 values from the same instrument and FVC maneuver from a different office spirometer (EasyOne®; ndd Medical Technologies, Andover, MA, USA) and found a correlation coefficient of 0.72.[26] Lundgren et al. used prebronchodilator values hence their finding did not address its utility in the diagnosis of COPD, which requires a Post Bronchodilator Value.[33]

The strength of our study was its prospective nature and all spirometric measurements were done by a single trained operator using single machine and all the values were taken from the single best effort, thus avoiding the effort to effort variation. Spirometry results were analyzed for their quality and acceptability.

4.1. *Limitations*

This was a single-center study done in south India hence our findings might not fit for the rest of the Indian population. As most of the subjects were males and smokers, the generalization of the results to non-smokers and females is doubtful.

5. Conclusion

This prospective cross-sectional study demonstrated a positive correlation coefficient ($r = 0.972$, $p < 0.01$) between the post-bronchodilator FEV1/FEV6 and FEV1/FVC ratios and a cut off value of 71.845 for the post-bronchodilator FEV1/FEV6 ratio ($p < 0.01$), which should be used as fixed diagnostic criteria for

COPD. Thereby, we conclude that the FEV1/FEV6 ratio should be used as a surrogate for the FEV1/FVC ratio in the diagnosis of Chronic Obstructive Pulmonary Disease. The specific use of the FEV1/FEV6 ratio for the diagnosis of COPD has the advantage of a fixed cut-off time and easier for the patient to perform.

With the validation of several handheld devices to measure the FEV6, now is the time to derive reference values and to arrive at a consensus for a fixed cut-off value of FEV1/FEV6 for the diagnosis of COPD.

Presentation at a meeting

NAPCON 2018 at Ahmedabad, India.

Conflicts of interest

The authors have none to declare.

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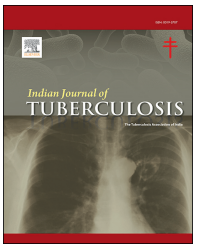

Original Article

Abdominal tuberculosis in a tertiary care centre in Saudi Arabia

Fatehi Elzein [a,*], *Razan Kharraz* [a], *Ayah Boudal* [a], *Haris Mohamed* [a], *Mohammed Mursi* [a], *Hadi Kuriry* [b], *Ali Albarrak* [a], *Nisreen AlSherbeeni* [a]

[a] *Infectious Diseases Unit, Prince Sultan Military Medical City (PSMMC), Riyadh, Saudi Arabia*
[b] *Hepatology Unit, Sultan Military Medical City (PSMMC), Riyadh, Saudi Arabia*

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ABSTRACT

Objectives: Abdominal tuberculosis (ATB) is the second most common type of extra-pulmonary tuberculosis. Though it does not usually pose a significant risk of infectivity, ATB can go unidentified and progress to disseminated infection. The aim of this study is to highlight the incidence and outcome of this infection in a tertiary care centre in the Kingdom of Saudi Arabia (KSA).

Methods: In this retrospective study, we included all ATB patients admitted to our centre between January 1 st, 2010 and December 31, 2018. A total of 42 patients with a median age of 49 (range 18–83 years, 78.6% males) were identified.

Results: The most common presentation was abdominal pain, weight loss, and abdominal distension. All the patients were HIV negative; however, 50% had a comorbid condition, mainly diabetes mellitus, chronic renal failure, and liver cirrhosis. Tuberculous peritonitis was the predominant type of ATB. Suspicious and potentially malignant abdominal masses appeared on the abdominal CT scans of six patients. This suggest that TB should be excluded in patients from endemic area presenting with abdominal masses.

All patients received standard anti-tuberculous medication for an average duration of 7.4 months. The outcome was excellent with 88%% achieving complete response. Adjunctive corticosteroids were not used, and none of the patients had a surgical complication.

Conclusion: The diagnosis of ATB is challenging. It can mimic inflammatory bowel disease in young populations and malignancy in middle-aged and elderly population. For this reason, a high index of suspicion with prompt treatment is required to improve the prognosis and prevent complications.

* *Corresponding author*. Dr. Fatehi E Elzein Infectious Diseases Unit, PSMMC Riyadh, P.O. Box 7897, Riyadh, 11159, Saudi Arabia.
E-mail address: fatehielzein@gmail.com (F. Elzein).

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1. Introduction

Extrapulmonary tuberculosis (EPTB) represents 15% of global cases tuberculosis (TB), and 24%–31% in the Eastern Mediterranean Region and Saudi Arabia.[1,2] A higher prevalence of EPTB is reported in patients with HIV co-infection.[3] In a recent review, TB lymphadenitis (58.1%) was the most frequently reported type followed by gastrointestinal (18.7%) and central nervous system TB (9.6%).[2] Overall, abdominal tuberculosis (ATB) accounts for 1.0–6.1% of all EPTB.[4] In Saudi Arabia Al Otaibi F. et al concluded that ATB comprises 13.3% of extra-pulmonary TB and affects young patients between 25 and 45 years of age. Both sexes are equally affected in their study.[5–7] Typically, ATB involves the gastrointestinal tract, peritoneum, lymph nodes, solid (liver, pancreas, and spleen), and pelvic organs. Though it does not usually pose a significant risk of infectivity, ATB can go unidentified and progress to a disseminated infection.

The objective of our study is to document the epidemiologic profile, diagnostic methods, and management outcomes of patients with ATB in a tertiary care setup. Identifying cases of ATB earlier may help reduce patient morbidity, mortality, and costs to the health care system.

2. Material and methods

This is a single-centre, record-based retrospective study of all ATB patients diagnosed between January 1st, 2010 and December 31, 2018 in Prince Sultan Military Medical City (PSMMC), Riyadh, Kingdom of Saudi Arabia (KSA). PSMMC is a large tertiary care centre consisting of 1200 beds. Data was obtained from the infectious disease consult database, TB coordinators, and microbiology and pathology laboratory registers. Patients' records were retrospectively reviewed for the following parameters: demographic data, clinical features, site of infection, laboratory and radiology results, treatment, follow-up duration, and clinical outcome.

The Institutional Review Board of PSMMC approved patient data review.

2.1. Statistical analysis

The Statistical Package for Social Sciences (SPSS Inc., IBM, New York, US) version 23 was used for data analysis. A Shapiro–Wilk test was used to evaluate normal data distribution. Frequencies and percentages were used to describe categorical data, while means and standard deviations (minimum–maximum) were used for normally distributed continuous data, or median (25–75 percentile) were used for abnormally distributed continuous data. A Mann–Whitney U and an unpaired student t-test were utilised to test differences between abnormally distributed variables, while a Pearson's chi-square test was utilised to test dependence between dichotomous categorical data that matched the 2×2 contingency assumptions. A P value < 0.05 was considered statistically significant.

2.2. Epidemiology and clinical findings

Of 635 individuals with TB diagnosed between 2010 and 2018, 42 (6.6%) patients fulfilled the criteria for ATB. Baseline demographic and clinical characteristics are presented in Table 1. Most patients were males (78.6%), with a mean age of 49.36 ± 19.72 (range of 18–83 years). The peritoneum is the focus of infection in 29 (69.0%) patients. Thirteen (31.0%) had an extraperitoneal disease; luminal and visceral disease were detected in six patients each (14.3%). The diagnosis was definite in 34 (81.0%) and presumptive in 8 (19.0%) of the patients. A definite diagnosis of ATB was based on the presence of ≥2 of the following criteria[1]: clinical, imaging, or endoscopic evidence of GI involvement[2]; AFB smear and/or culture positive fluids or biopsies; and/or[3] caseating granuloma.in the same way, while presumptive diagnosis is made on the presence of a strong clinical suspicion, typical radiologic, and histological features that are confirmed by a persistent response to treatment. Of note two patients had concomitant pulmonary TB, while in one patient both ATB and Crohn's disease coexisted. A human immunodeficiency virus (HIV) test was negative in tested patients (39/42). However, 21 patients (50.0%) had a comorbid illness with diabetes mellitus (DM) in 34.1.0%, chronic renal failure 12.0% and liver cirrhosis 4.7%. Common symptoms at presentation were abdominal pain (69.0%), weight loss (57.1%), fever (45.2%), and abdominal distension (40.55%) (Table 1). Malignancy was suspected in six (14.3%) patients who presented with abdominal mass.

2.3. Radiology and endoscopic findings

The chest x-ray was normal in 71.4%. Ninety-five percent of the patients underwent a computer tomography (CT) scan of

Table 1 – Demographic characteristics, presentations, and investigations of patients with ATB (n = 42).

Characteristics	n (%)
Age (years) Mean ± SD	49.36 ± 19.72
Male	33 (78.6%)
Presenting symptoms and signs	
Abdominal pain	29 (69.0%)
Weight loss	24 (57.1%)
Fever	19 (45.2%)
Ascites	17 (40.55)
Anorexia	15 (35.7%)
Changes in bowel habits	12 (28.6%)
Abdominal mass	6 (14.3%)
Splenomegaly	4 (9.5%)
Peritonitis	3 (7.1%)
Hepatomegaly	3 (7.1%)
Rectal bleeding	2 (4.8%)
Location	
Peritoneal	29 (69.0%)
Extra-peritoneal	13 (31.0%)
Multicentre	2 (4.8%)
Luminal	6 (14.3%)
Visceral -Liver, pancreas, spleen etc.	6 (14.3%)
Investigations	
Hemoglobin level (g/dl)	11.57 ± 1.99[8–17]
Serum albumin levels (g/L)	35.02 ± 6.25
C- reactive protein (CRP) mg/L	37.00 (12.00–113.00)
Erythrocytic sedimentation rate (ESR) (mm/hr.)	17.50 (6.50–48.75)
CA- 125 levels (U/ml)	137.00 (24.00–267.00)
Serum vitamin D level (nmol/L)	26.00 (16.00–41.00)
Serum creatinine level (umol/L)	77.00 (56.75–106.00)

the abdomen. Peritoneal thickening and/or omental caking were seen in (76.2%). Moreover, eight patients (19%) had intrabdominal masses, including three pancreatic and two ovarian lesions, while terminal ileum thickening was detected in five (12.5%) patients. One patient had a right common iliac artery aneurysm. Almost all patients with peritoneal TB showed omental caking or peritoneal thickening on CT scan, while 41.4% had ascites. A laparoscopy/laparotomy performed on 31 (73.8%) of the patients revealed omental nodules in 48.3% of peritonitis patients. Eight patients had visceral adhesions (Table 2).

2.4. *Laboratory findings*

The main laboratory findings in our cohort include anaemia in 66.7% and elevated CRP and ESR in 83.3% and 52.4%, respectively (Table 3). Both vitamin D and albumin were low in 54.8% and 40.5%, respectively. Cancer antigen 125 (CA-125) was tested in seventeen out of 42 patients. Thirteen of these patients were males. The mean CA-125 level was 137.00 U/ml with abnormal CA-125 level in 71.0% of the tested patients. Remarkably CA-125 declined following treatment in most of the patients (Fig. 1). Histopathology done in 39 of 42 patients (92.8%) disclosed a granuloma in all the patients. Conversely, MTB culture was positive in only 17 out of 42 (40.5%) of the tissue cultures. Likewise, MTB was isolated in only 33% of ascitic fluid cultures. All MTB isolates were fully sensitive except for two with rifampicin resistance.

Table 2 – Radiological and endoscopic findings in ATB patients (n = 42).

Procedure	All Cohort	Peritoneal location (n = 29)	Extra peritoneal location (n = 13)	p-value
Chest X ray (n = 42)				
Abnormal	12 (28.6%)	10 (34.5%)	2 (15.4%)	
Computer Tomography (CT) (n = 42)				
Nodal enlargement (LN)	22 (52.4%)	15 (51.7%)	7 (53.8%)	0.088
Peritoneal thickening	17 (40.5%)	15 (51.7%)	2 (15.4%)	0.002
Omental caking	15 (35.7%)	14 (48.3%)	1 (7.7%)	0.001
Ascites	13 (31.0%)	12 (41.4%)	1 (7.7%)	0.002
Visceral thickening	11 (28.3%)	4 (13.8%)	7 (53.8%)	0.366
Masses	8 (19.0%)	5 (17.2%)	3 (23.1%)	0.480
Abscess	2 (4.8%)	2 (6.9%)	–	–
Others	12 (28.6%)	4 (13.8%)	8 (61.5%)	0.248
Laparoscopy – (n = 31)				
Peritoneal/ Omental deposits/ nodules	17 (54.8%)	14 (48.3%)	3 (23.1%)	0.008
Ascites	14 (45.2%)	12 (41.4%)	2 (15.4%)	0.008
Laparoscopy not done	11 (35.5%)	7 (24.1%)	4 (30.8%)	0.366
Visceral adhesions	8 (25.8%)	6 (20.7%)	2 (15.4%)	0.157

Table 3 – Diagnostic tests in all patients with ATB (n = 42).

Procedure	Positive	Negative	Not done (ND)
MTB culture n (%)	17 (40.5%)	24 (57.1%)	1 (2.4%)
PCR n (%)	2 (4.8%)	21 (50.0%)	19 (45.2%)
AFB n (%)	5 (11.9%)	33 (78.6%)	4 (9.5%)
Histopathology (Granuloma) n (%)	39 (92.8%)		3 (7.2%)

2.5. *Treatment*

Forty patients were started on an initial two-month phase of drugs including isoniazid, rifampicin, pyrazinamide, and ethambutol followed by two drugs (isoniazid and rifampicin) for a minimum treatment duration of 6 months. Their course was complicated by drug-induced liver injury (DILI) in seven patients. DILI is defined as a rise in liver enzymes 3 times above upper limit of normal (ULN) with symptoms or 5 times ULN without symptoms. Thirty-four (81.0%) patients completed a mean follow-up of 16.7 (6.0–60.0) months. The outcome of treatment was excellent in 88% and none of the patients had evidence of disease recurrence. Overall, four patients died: two before initiation of treatment and two near or after completion of therapy (Table 4).

3. Discussion

In this retrospective study we describe a cohort of patients with ATB from a country of a moderate TB burden. None of the patients were co-infected with HIV, so our findings might not apply to areas of high HIV co-infection. Compared to previous studies, our patients were relatively old and mostly men (78.0%).[2] Similar to in earlier studies, abdominal pain was present in 61.0% of the patients.[8] However, fever was detected in fewer than half of the subjects. Consequently, three of our patients were suspected for pancreatic malignancy, while a fourth patient was diagnosed via radiology as metastatic gastric carcinoma. Primary gastric TB is rare (0.4%–2.0%) and can mimic gastric carcinoma.[9,10] Gastric tuberculosis is described more commonly in males than females with a ratio of 2.8:1.[11] Likewise, pancreatic TB is exceedingly rare. Additionally, there are no distinctive features to differentiate pancreatic TB from pancreatic carcinoma.[12] This underscores the importance of excluding TB in such presentations, particularly in endemic areas.

ATB remains a diagnostic and therapeutic challenge. In resource-poor countries therapeutic trials of ATT may be justified. In such cases clinical suspicion and favourable responses to treatment can support a clinical diagnosis of ATB. However, a prolonged follow-up is required. It is worth mentioning that ATB can mimic carcinomatosis in the elderly and inflammatory bowel disease (IBD) in young patients. In some countries the incidence of ATB is found to be similar to that of IBD, which makes the former a challenging diagnosis to rule out in such patients.[8] Occasionally and like for one of our patients both Crohn's disease and TB may coexist. Therefore, microbiologic, and/or molecular diagnosis of ATB is essential to avoid missing ATB.

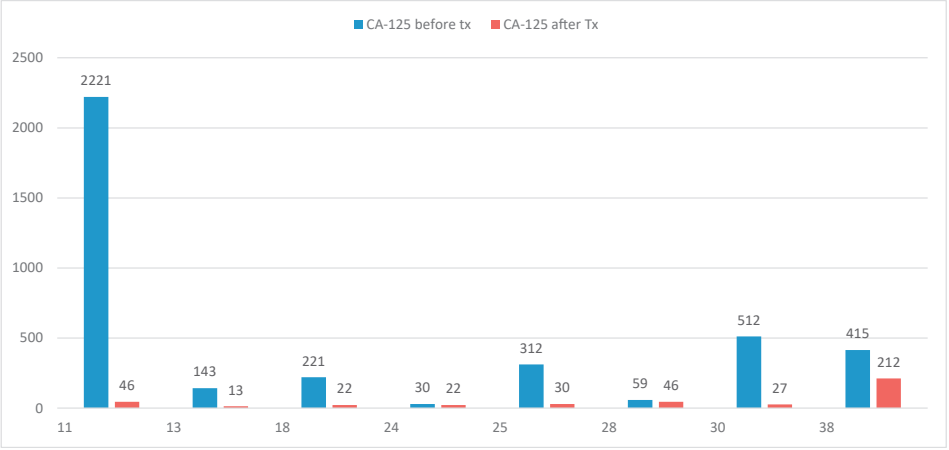

Fig. 1 – CA-125(U/ml) before and after treatment in eight patients.

Table 4 – Diagnosis, treatment, and outcome in patients with ATB (n = 42).

	n (%)
Diagnosis (n = 42)	
Definitive	34 (81.0%)
Presumptive	8 (19.0%)
Treatment and outcome (n = 42)	
Conventional ATT	40 (100%)
Treatment duration (months) mean ± SD	7.41 ± 1.81
Drug induced liver injury	7 (17.5%)
Follow-up for 1-year	34 (81.0%)
Average Duration of follow-up (months)	16.74 ± 12.69
Death	4 (9.5%)
Response	
Complete	37 (88.1%)
Partial or no response	2 (4.8%)
lost follow-up	1 (0.5%)

ATT = anti-tubercular treatment.

In accordance with previous studies, all our patients with peritoneal TB were suspected via CT scan of the abdomen. In addition, eight (24.0%) of the patients had abdominal/pelvic masses or abscesses requiring further evaluation. A diagnosis of pelvic TB can be demanding. Erroneous diagnosis of malignancy can be made on initial assessment. However, A new abdominal compartmentalization sign where the abdominal cavity was separated into compartments by adhesions was observed by Sharma in 18 (10.73%) cases.[13] In the current study a young patient was planned for possible salpingo-oophorectomy following the finding of an ovarian cyst and a CA-125 of 2221 U/ml. Laparoscopy was suggestive of extensive metastasis involving the whole abdomen including the omentum, uterine fundus, abdomen, and pelvic peritoneum. However, tissue obtained via a mini laparotomy was positive for MTB. The extremely high CA-125 declined to 16 U/ml while on treatment. This rise in CA125 was unusual since a level greater than 1000 is postulated to indicate ovarian malignancy rather than TB.[14] In one study 26 females with a pelvic mass and elevated CA125 underwent a laparotomy or laparoscopy for subtotal hysterectomy-oophorectomy and salpingectomy for a presumed malignancy. Their pathology confirmed TB, and they were cured with anti-tuberculous therapy.[15] Remarkably 76.5% of our patients with a high CA-125 were males. Of note, CA-125 was reported to be high in about 40% of pulmonary TB cases and in a large number peritoneal TB cases.[5,16] Although CA125 might not be of a great diagnostic value, its decline on anti-tuberculous drugs remains a reliable indicator of response to treatment.[17]

In the current study most of the patients are immunocompetent. However, 50% of the patients had a comorbid illness including DM, CRF and liver cirrhosis. Diabetes mellitus is a recognised significant risk factors for the development of TB. Furthermore, defective cellular immunity seen in CRF patients, predisposes to *M. tuberculosis* infection. Equally, cirrhotic patients are 15 times more likely to develop TB, particularly peritoneal TB, than the general population.[18] Strikingly, 50.0% of our patients had vit D deficiency (VDD). Data is lacking regarding the role of vitamin D in ATB; however, a recent study showed similar findings, with VDD being common in ATB.[19] Vitamin D is recognised as playing a role in the immune response to TB and other inflammatory disorders. Furthermore, an association between vitamin D receptor gene (VDR) with pulmonary tuberculosis has been previously described.[20] Patients with VDD may have an increased risk of acquiring pulmonary TB, nonetheless the effect of supplementation with vitamin D on the outcome of TB is controversial.[21]

The diagnosis was established by diagnostic laparoscopy in 73.8% of our patients. Nevertheless, laparoscopy may not be feasible in all centres. Accordingly, endoscopic ultrasound (EUS) emerged as a non-invasive alternative. A study showed that EUS fine needle aspiration (FNA) sensitivity and specificity is almost 100% in diagnosing tubercular lymphadenopathy.[22] In the current study a definitive diagnosis was made in 39 out of 42 patients (92.8%). Nine patients (23.07%) had caseating granuloma, while non-caseating/necrotising granuloma was seen in 71.4% of the patients. Caseating necrosis in granulomas is the histologic hallmark of TB; nevertheless, it is infrequently found. Importantly the presence of granuloma does not make the diagnosis, while its absence does not rule it out. Both IBD and malignancy can be associated with granuloma. Moreover, well-formed granulomas are not detected in all of the cases of TB, with only 20%–54% cases demonstrating

established granulomas.[4,23] Therefore tissue and/or ascites MTB culture should be obtained in all cases. Worth mentioning, ascitic fluid culture for AFB and direct smears have a poor sensitivity.[24,25] In this study 17 out of 41 (41.5%) patients had culture positive MTB from biopsy specimens. However, a lower ascitic fluid culture yield of 33.0% was similar to previous reports.[26]

Abdominal tuberculosis is generally managed medically with surgery being reserved for complications. Like for other types of TB, six-month treatment is usually adequate in all ATB types. Multiple studies showed there was no difference in the outcome between antituberculosis therapy delivered for either six months or nine months for ATB.[27,28] The quality of evidence suggesting the benefits of steroids in ATB is poor and cannot be generalised.[29] None of our patients required steroid treatment. In keeping with other trials, the outcome of our patients was excellent with 88.0% achieving a complete response. However, recurrence of the disease remains a concern, and prolonged follow-up is required. None of our patients had a recurrence despite an average follow-up of 17 months.

4. Conclusion

Abdominal tuberculosis usually presents with non-specific symptoms. It can be confused with IBD or intrabdominal malignancy. The diagnosis can be challenging particularly in non-endemic areas. A delayed diagnosis is associated with an increased risk of complications. Hence, a high index of suspicion is required to make an early diagnosis and initiate prompt treatment.

Conflicts of interest

None.

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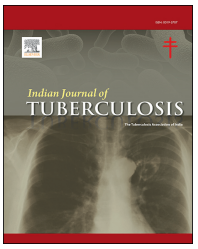

Original Article

Evaluation of real time polymerase chain reaction targeting *mpb64* gene for diagnosis of extrapulmonary tuberculosis

Kavita Vijay Chaudhari [a], *Pampa Ch Toi* [b], *Noyal Mariya Joseph* [a,*]

[a] *Department of Microbiology, JIPMER, Puducherry, India*
[b] *Department of Pathology, JIPMER, Puducherry, India*

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ABSTRACT

Background: Paucibacillary nature of extrapulmonary tuberculosis (EPTB) has paved way for molecular methods increasingly being used for diagnosis. We undertook a study for evaluation of sensitivity and specificity of real-time polymerase chain reaction (RT-PCR) targeting *mpb64* gene for diagnosis of EPTB.

Methods: A total of 152 clinical samples from suspected cases of EPTB were included in this study. All samples were extracted using spin column based commercial DNA extraction kit and were subjected to RT-PCR targeting *mpb64* and IS6110. Smear and culture was also done for samples whenever quantity was sufficient. Cytology report was noted from hospital information system. Receiver operating characteristic (ROC) curve analysis was done for determining cut-off Ct value for *mpb64* RT-PCR. Melt curve analysis was done for samples whose cycle threshold (Ct) value was more than 37. The sensitivity and specificity of the *mpb64* RT-PCR was calculated using a composite gold standard i.e., positive for one or more of the following: microscopy (including fine needle aspiration cytology (FNAC), acid-fast bacilli positivity), culture and IS6110 RT-PCR.

Results: Out of the 152 samples, 72 (47.4%) were positive for tuberculosis by composite gold standard. Samples consisted of ascitic fluid (12), CSF (35), pus (23), lymph node aspirate (35), pleural fluid (37), synovial fluid (4), urine (1), pericardial fluid (1) and tissue bits (4). Microscopy (AFB smear including lymph node aspirate) was done for 124 samples of which 43 (34.7%) were positive. Culture results were available for 79 samples, 25 (31.6%) of which were positive and 42 (27.6%) of the 152 samples were positive by IS6110 PCR. Based on ROC and melt curve analysis, *mpb64* RT-PCR was able to detect 38 (52.8%) of the 72 positive samples. In comparison to IS6110 RT PCR, 4 additional cases were detected by *mpb64* RT-PCR. Compared to composite gold standard *mpb64* showed overall sensitivity of 52.8%.

Conclusion: The *mpb64* RT-PCR is highly specific or MTB and can be used as a supplemental test for diagnosis of EPTB along with other diagnostic tests. However the overall sensitivity

* *Corresponding author*. . Tel.: +91 9843094673
E-mail address: noyaljoseph@yahoo.com (N.M. Joseph).
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of *mpb64* RT-PCR is too low to be used as an independent test for diagnosis of EPTB. Combining the results of IS6110 RT PCR and *mpb64* RT PCR improved the overall sensitivity and hence *mpb64* can be used as an additional target for diagnosis of EPTB.

1. Introduction

Extrapulmonary tuberculosis (EPTB) comprises of 15% of total tuberculosis patients in the world.[1] Unlike classical features of pulmonary TB (PTB), extrapulmonary disease has vague manifestations. The exact numbers of EPTB cases that are diagnosed represent only the tip of an iceberg. Paucibacillary nature of EPTB samples has hindered the development of a rapid and sensitive test. In spite of good laboratory practices sensitivity of conventional tests for detecting EPTB still remains significantly low. Molecular tests reduce the turnaround time for diagnosis of EPTB, thus ensuring faster commencement of anti-tubercular therapy and earlier cure.[2,3] Xpert MTB/RIF a cartridge based polymerase chain reaction (PCR) test targeting *rpoB* gene is widely used for diagnosis of pulmonary TB. However the sensitivity of Xpert MTB/RIF for diagnosing EPTB is variable owing to paucibacillary nature of EPTB and wide variety of specimen types. Although it is useful in early diagnosis of certain EPTB types like lymph node TB, it is not a recommended for diagnosis of pleural TB due to its low sensitivity.[1] Various gene targets have been evaluated for their sensitivity and specificity in diagnosis of EPTB.[3] The IS6110 target has been thoroughly studied and provides an additional advantage of being present in multiple copies in the genome of *Mycobacterium tuberculosis* complex (MTBC). However, in certain strains of MTBC, where IS6110 gene is absent, such a single gene targeted assay might lead to a false negative result.[4] Another gene of interest is the *mpb64* gene, which codes for Mpt64 protein of MTBC. It has been evaluated along with *IS6110*, by many researchers and is found to have higher sensitivity and specificity, when used in combination with other target genes, for diagnosis of EPTB cases.[3,5,6]

The present study was undertaken to evaluate the role *mpb64* gene as a single gene in diagnosing EPTB using real time PCR. Due to technical difficulties in diagnosing EPTB combine gold standard consisting of smear, culture and real time PCR using IS6110 gene was used.

2. Materials and methods

The present diagnostic study was conducted in Department of Microbiology, JIPMER Puducherry from January 2017 to November 2018. Extrapulmonary samples from suspected cases of EPTB satisfying the inclusion and exclusion criteria were included in the study. This study was approved by Institutional Ethics committee and waiver of consent was obtained(JIP/IEC/2016/1039).

The sample size for estimating sensitivity of real time polymerase chain reaction targeting *mpb64* gene, assuming a sensitivity of 90%, prevalence of extrapulmonary tuberculosis as 10% and a relative precision of 15% with 95% confidence level was calculated to be 152.

Extrapulmonary specimens from clinically suspected cases of EPTB received in the department of Microbiology for *M. tuberculosis* PCR were included in study. Samples from patients on anti-tubercular treatment were excluded. All samples were collected in screw capped sterile 50 mL polypropylene centrifuge bottles. All specimens were refrigerated at 2–8 °C in laboratory until further processing. Real time PCR targeting IS6110 and *mpb64* was done for all samples. Samples with quantity more than 1 mL was divided into two aliquots, of which one was for real time polymerase chain reaction (aliquot 1) and other was utilized for culture and smear (aliquot 2). Lymph node aspirate was simultaneously sent for cytological examination and report was noted from hospital information system.

2.1. *Real time polymerase chain reaction*

2.1.1. *DNA extraction*

The aliquot 1 meant for real time polymerase chain reaction was subjected to DNA extraction using **HELINI™ Purefast Bacterial Genomic DNA Minispin prep Kit (Helini Biomolecules Chennai).** Extraction protocol was according to manufacturers' instruction. Positive (MTB H37Rv) and negative control (Milli Q water) were included in each batch of extracted specimen. Sample was centrifuged at 8000 rpm for 5 min. and 200 μL of sediment was collected in a sterile microcentrifuge tube. 180 μL of Digestion buffer and 20 μL of Lysozyme was added to the tube. In case of tissue sample, 5 gm. of tissue was homogenized using sterile mortar and pestle and then 200 μL of tissue lysis buffer was added to it and incubated for 45 min. at 56 °C in water-bath vortexing in-between till complete lysis. After incubation, 200 μL of Binding Buffer and 20 μL of Proteinase K was added to microcentrifuge tube. 5 μL of internal control template (synthetic DNA) was added and tube was vortexed and incubated at 56 °C for 15min. in dry thermal block. Molecular grade absolute ethanol 200 μL was added to the microcentrifuge tube and mixed well. Entire sample was pipetted into Purefast® spin column and was centrifuged at 8000 rpm for 1 min. The flow-through was discarded and spin column was place back into new sterile collection tube. To the spin column 500 μL of reconstituted Wash buffer-1 was added and centrifuged at 10000 rpm for 1 min and flow-through was discarded. Spin column was placed back into new collection tube. To the spin column 500 μL of reconstituted Wash buffer-2 was added and centrifuged at 14000 rpm for 3 min and the flow-through was discarded. Spin column was placed into new collection tube. Empty spin was given at 14000 rpm for 1 min. Spin column

was transferred into a fresh 1.5 mL microcentrifuge tube and 100 μL of the pre-warmed Elution Buffer was added to the center of spin column membrane. It was incubated for 2 min at room temperature and centrifuged at 8000 rpm for 1 min. Spin column was discarded and purified DNA was labeled appropriately. The extracted DNA was divided into two aliquots (A & B) and stored in a deep freezer at −20 °C until further processing. Aliquot A and B were utilized for real time PCR targeting IS6110 and real time PCR targeting *mpb64* respectively.

2.1.2. *Real time polymerase chain reaction targeting IS6110*

Real time PCR assays for IS6110 and *mpb64* were performed on Light cycler COBAS z 480 analyzer (Roche Molecular Diagnostics, USA). Real time PCR targeting insertion sequence IS6110 was performed using a commercial kit **HELINI™ MTB Real time PCR kit (Helini Biomolecules Chennai)**. Test was performed according to manufacturers' instructions. Detection limit of kit was 10 copies/ml according to manufacturers' instruction manual.

2.1.3. *Real time polymerase chain reaction targeting* mpb64

The primer and probe sequences were same as described by Pinhata et al.[7] Primers −probe mix were prepared by **Helini Biomolecules Chennai**. Reaction volume of 25 μL was used which consisted of 10 μL of sample DNA extract along with 12.5 μL TaqMan Master Mix (HELINI™) along with 2.5 μL primer probe mix. Cycling conditions consisted of enzyme activation at 95 °C for 15 mins. 45 cycles each consisting of denaturation for 20 seconds at 95 °C, annealing for 20 seconds at 56 °C, and extension for 20 seconds at 72 °C. Amplified products were detected by the use of TaqMan probes (FAM-TATCGATAGCGCCGAATGCCGG-BHQ1) labeled at the 5′ position with FAM and at the 3' position with BHQ1. A synthetic non human gene was used as internal control for extraction and amplification. Internal control was detected at HEX channel.

2.2. *Standardization of PCR*

Validation of PCR was done using known positive and negative sputum samples and also using H37Rv spiked samples. 0.5 McFarland standard was prepared using cultures of H37Rv strain and was serially diluted to achieve concentrations of 10^8,10^7,10^6,10^5,10^4,10^3,10^2 and 10^1 bacilli/ml which were then spiked into known negative samples. These spiked samples were extracted and real time PCR targeting *mpb64* was performed. The limit of detection of *mpb64* real time PCR was found to be 100 copies/mL. H37Rv DNA was used as positive control, nuclease free water was used as negative control for PCR and synthetic heterologous DNA was used as internal (amplification) control.

2.3. *Melt curve analysis*

Melt curve analysis was done for samples whose *mpb64* real time PCR C_t value was >37 cycles. Melt curve was performed on **Applied Biosystems™ QuantStudio 5™** Real-Time PCR System (ThermoFisher Scientific, USA). A reaction volume of 20 μL consisted of 10 μL Mastermix **(Takyon™ Low Rox SYBR® MasterMix dTTP blue (EuroGentec))**, 4 μL of DNA template, 2 μL of each forward (GTGAACTGAGCAAGCAGACCG) and reverse (GTTCTGATAATTCACCGGGTCC) *mpb64* primer and 2 μL of nuclease free water. Melt curve was standardized using H37Rv DNA extract as positive control and nuclease free water as negative control. The melting temperature T_m was determined to be 83.9 ± 0.5. The samples with similar melting temperature were considered as positive for presence of *mpb64* gene.

2.4. *Culture and smear*

Sterile body fluids were processed without any decontamination procedure. Pus samples were subjected to decontamination process using NALC- 4% NaOH method. Tissue samples were homogenized using mortar and pestle and decontaminated using NALC-NaOH method. After centrifugation sediment was inoculated into Lowenstein Jensen medium (LJ) and Mycobacteria Growth Indicator Tube (MGIT) as per standard protocol for culture. Smear was prepared from remaining part of sediment and stained by Ziehl Neelsen stain. All LJ culture bottles with growth and MGIT culture tubes flagged as positive by MGIT 960 were subjected to Ziehl Neelsen staining and examined for the presence of acid fast bacilli. The culture isolates were identified as *M. tuberculosis* complex based on immunochromatographic test for MPT64 antigen, colony morphology (dry, rough, trough, buff coloured colonies on LJ media) and granular turbidity in MGIT tube.

3. Results

Extrapulmonary samples from a total of 152 patients were included in this study. Out of these 152 samples, 72 (47.4%) were positive for tuberculosis (TB) by the composite gold standard (microscopy (including fine needle aspiration smear), culture, and real-time PCR targeting IS6110). Pleural fluids, CSF, lymph node aspirate (LNA) and pus were the common extrapulmonary samples sent for diagnosis of EPTB (Table 1).

3.1. *Results of smear and culture*

Of the 152 samples, screening for AFB in smear or lymph node aspirate cytology (LNA) was done for 124 samples and IS6110 real-time PCR was performed for 152 samples. Culture was done for only for 94 samples as the specimen volume were insufficient for the remaining. Of the 94 cultures, 15 were

Table 1 – Specimen types included in study.

Sample type	Frequency (n = 152)
Pleural fluid	37
CSF	35
Lymph node	35
Pus	23
Ascitic fluid	12
Synovial fluid	4
Tissue bit	4
Urine	1
Pericardial fluid	1

Table 2 – Results of the different diagnostic tests included in the composite gold standard.

Test	Positive	Negative
AFB in smear/LNA (n = 124)	43 (34.7%)	81 (65.3%)
Culture (n = 79)	25 (31.6%)	54 (68.4%)
IS6110 RT-PCR (n = 152)	42 (27.6%)	110 (72.4%)

LNA-lymphnode aspiration cytology.

contaminated, so culture results were available for only 79 samples. The results of the AFB screening, culture and IS6110 real-time PCR are shown in Table 2.

3.2. *Results of real -time PCR targeting* mpb64

The *mpb64* real-time PCR showed amplification curve for 43 of the total 152 samples.

Of these 17 (39.5%) samples had a C_t (threshold cycle) value ≤ 35 (Fig. 1). The remaining 26 (60.4%) samples had C_t (threshold cycle) value between 36 and 40.

3.2.1. *Determination of cut-off for* mpb64 *real-time PCR*

Receiver Operating Characteristic (ROC) curve analysis was performed for determining the cut-off for *mpb64* real-time PCR. The area under the curve (AUC) was 0.806 (95% CI, 0.732 to 0.880) (p value < 0.001). Based on the coordinates of the ROC curve, the cut-off was decided to be Ct value of 38, for which the specificity was 100% and the sensitivity was 45.8%. Therefore, in this study the samples showing an amplification curve less than 38 were considered to be true positive.

3.2.2. *Melt curve analysis of samples positive by* mpb64 *real-time PCR*

The melt curve analysis was initially done for three samples which showed amplification curve with Ct values between 37 and 38 in *mpb64* real-time PCR. All three samples showed a melting temperature similar to that of a known true positive (Fig. 2). This confirmed that the cut-off value of 38 as determined by ROC curve analysis can be accepted as true positive. Melt curve analysis was then done for samples showing amplification curve with Ct values between 38 and 40. Only six of these samples were confirmed to be true positive by melt curve analysis. However, other samples with Ct values between 38 and 39 and all of the samples with Ct values 40, were revealed to be false positive by melt curve analysis.

3.2.3. *Positivity of* mpb64 *real-time PCR*

Based on our ROC curve analysis and melt curve analysis, samples with Ct value ≤ 38 or positive by melt curve analysis (if CT value above 38) were considered as positive. Accordingly out of 152 samples, 38 (25%) were positive by *mpb64* real time PCR.

3.3. *Sensitivity and specificity of* mpb64 *real-time PCR compared to composite gold standard*

Of the 72 patients confirmed to have TB by composite gold standard, 38 (52.8%) were positive by *mpb64* real-time PCR. Of the 25 patients confirmed to have TB by culture, 13 (52.0%) were positive by *mpb64* real-time PCR. The sensitivity and specificity of *mpb64* real-time PCR were 52.0% and 96.3% respectively. Two samples negative by culture were positive by *mpb64* real-time PCR, but these samples were positive by IS6110 real-time PCR also. So, these two samples could have been either missed by culture or they would have had non-viable MTB. Sensitivity of different diagnostic tests for TB compared to composite gold standard is shown in Table 3. Sensitivity and specificity of *mpb64* real time PCR compared to composite gold standard for different specimens is shown in Table 4.

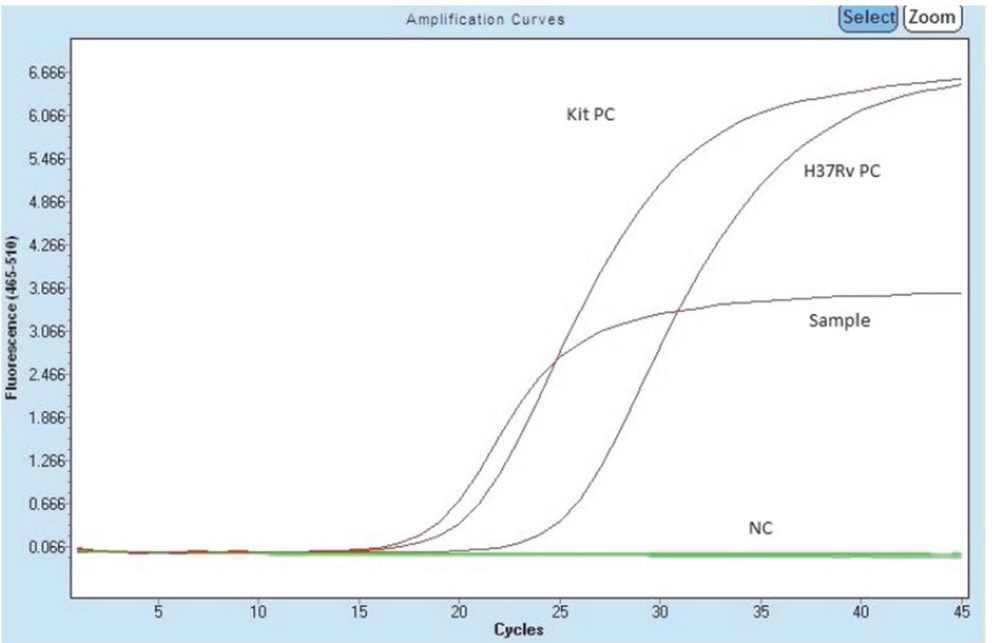

Fig. 1 – *Mpb64* real time PCR curve of a positive sample with along with positive. control (H37Rv), Kit positive control and negative control.

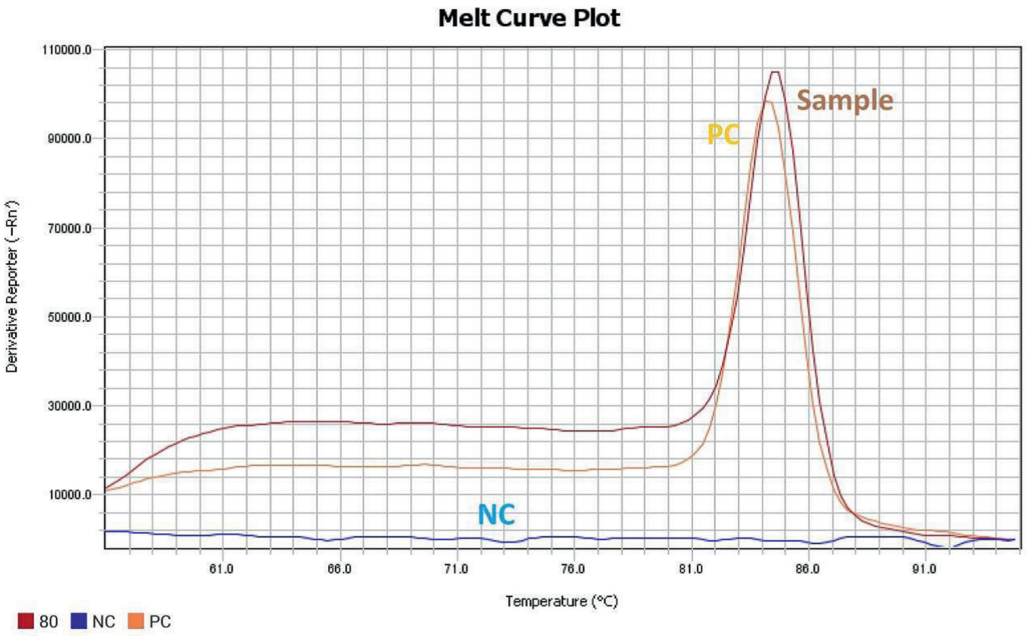

Fig. 2 – Melt curve analysis of a sample with true positive amplification curve.

3.4. *Comparison of IS6110 and* mpb64 *real-time PCR assays*

A total of 46 samples were positive by either IS6110 or *mpb64* real-time PCR assays or both. Of these 46 samples, 38 (82.6%) were positive by *mpb64* real-time PCR, while 42 (91.3%) were positive by IS6110 real-time PCR (p value 0.388). The *mpb64* real-time PCR detected 4 (8.7%) additional cases of TB which would have been otherwise missed by IS6110 real-time PCR. The combined sensitivity of both real-time PCR assays was 63.9%.

4. Discussion

Lack of highly sensitive test, vague clinical manifestations and paucibacillary nature of disease make diagnosis of EPTB difficult. Although various molecular targets have been tried so far none have been found good enough to be used as a solo target for diagnosing EPTB.[8–10] In previous studies conducted by researchers using conventional multiplex PCR targeting *mpb64* gene have shown good sensitivity and specificity for diagnosis of EPTB.[10] In Brazil, Martins et al conducted a study in which 73 EPTB samples were subjected to conventional nested PCR targeting *mpb64* gene which gave a sensitivity of 70% and specificity of 88%.[11] Using multiplex SYBR green RT-PCR Raveendran et al found sensitivity of *mpb64* to be 65.5% and specificity of 97.7%.[12]

In this study the sensitivity of *mpb64* RT-PCR was 52.8% when compared to combined gold standard. When sensitivity of *mpb64* RT-PCR was compared to culture positivity, it was found to be 52%. Pinhata et al got sensitivity of 90.3% in their study based on TaqMan RT-PCR targeting *mpb64* gene.[7] The higher sensitivity in their study can be explained by the fact that in pulmonary samples bacterial load is high, hence positivity is high.

In our study we found that among different specimen evaluated by *mpb64* RT-PCR, single specimen type which gave highest sensitivity was pus (76.5%) followed by lymph node aspirates (46.7%) (Specimen types with number less than 10, were not considered). High positivity was also found in pus (88.8%) and lymph node aspirate (89%) in study by Sharma et al.[14] The sensitivity of *mpb64* for diagnosing EPTB in pleural fluid in our study was 33.3% which was similar to that of Genexpert observed by Sharma S *et al*,[10] however in their study conventional multiplex PCR targeting *IS6110* and *mpb64* showed sensitivity of 89.6%.[10] Sensitivity of *mpb64* RT-PCR for CSF sample was 16.7%, which was less than that found in other studies.[15,16] Possible reason for decrease sensitivity can

Table 3 – Sensitivity of different diagnostic tests for TB compared to composite gold standard.

	Smear (n = 94)	Culture (n = 79)	IS6110 real time PCR (n = 152)	*mpb64* real time PCR (n = 152)
Sensitivity	36.8	73.5	58.3	52.8

Table 4 – Sensitivity and specificity of *mpb64* real time PCR compared to composite gold standard for different specimens.

Specimen	Sensitivity	Specificity
CSF (N = 35)	16.7%	100%
Pleural Fluid (N = 37)	33.3%	100%
Lymph Node Aspiration (N = 35)	46.7%	100%
PUS (N = 23)	76.5%	100%
Others (N = 22)[a]	85.7%	100%

[a] Ascitic fluid, urine, pericardial fluid, synovial fluid, tissue bit.

be less sample volume available for analysis. Another possible reason for low sensitivity can be due to tendency of MTB to form clumps in specimen due to which there is uneven distribution of bacteria in specimen.

In our study cutoff of C_t value 38 was determined for *mpb64* RT-PCR based on Receiver Operating Characteristic (ROC) curve plotting and melt curve analysis. In study by Palomo et al median Ct value for *mpb64* RT-PCR was 35.07.[13] Reasons for higher C_t value of cutoff in this study could be decreased bacterial load in sample or DNA degradation due to repeated freeze thawing during storage process. The possibility of PCR inhibition can be ruled out based on satisfactory internal control amplification. Melt curve analysis for presence of *mpb64* gene, of samples having C_t values between 38 and 40 revealed six positives. The possible explanation for this result can be poor binding of TaqMan probe and primer dimers interfering with amplification at late cycles.[17,18] We suggest that when using *mpb64* gene as target in RT-PCR, melt curve analysis can used to differentiate true positives and false positives at higher C_t values.

Of the 46 samples detected by RT-PCR, IS6110 was able to detect 91.3% (42/46) while *mpb64* detected 82.6% (38/46). The higher detection by IS6110 can be explained by its occurrence in multiple copies in MTB genome. *Mpb64* gene is present in single copy in the genome. However some isolates of MTB lack IS6110 gene which was also observed in our study as *mpb64* was able to detect 4 additional cases which were negative by IS6110 PCR.

Even though IS6110 is a more sensitive target, when used as single target it can potentially miss MTB cases (found to be 8.7% in our study). We observed that combining the results of *mpb64* RT-PCR to IS6110 RT-PCR results increased the sensitivity of RT-PCR from 52% to 63.9%.

Strength of our study- This is one of the few studies to used TaqMan probes based real time PCR using *mpb64* gene. To the best of our knowledge this is the first study to evaluate extrapulmonary TB using TaqMan probe for targeting *mpb64* as a single target. In our study, we have used a composite gold standard comprising of smear, culture and IS6110, to have microbiological confirmation of all the EPTB cases and facilitate reproducibility and comparison of the study findings in future. We have used appropriate sample size for good extrapolation of results in general population.

Limitations - We have not done quantification of MTB load in the EPTB cases which is one of the limitations of this study. Also it was not possible to do culture in few cases due to sample insufficiency.

5. Conclusion

The *mpb64* RT-PCR was highly specific for MTB and therefore can be used as a supplemental test for diagnosis of EPTB along with other diagnostic tests. The sensitivity of *mpb64* RT-PCR was higher for lymph node aspirates and pus samples compared to body fluids, so it may be useful for diagnosis of EPTB from these samples. The overall sensitivity of RT-PCR targeting *mpb64* gene was too low to be used as an independent target in diagnostic tests. However, the sensitivity of *mpb64* RT-PCR was comparable to that of IS6110 RT-PCR among the culture positive EPTB cases. Combining the results of IS6110 and *mpb64* RT-PCR improved the overall sensitivity, and therefore *mpb64* can be used as an additional target along with IS6110.

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Conflicts of interest

All authors have none to declare.

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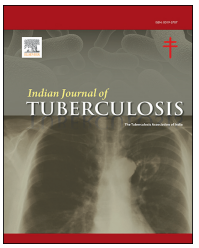

Original Article

Clinico-radiological profile and treatment outcome of pulmonary tuberculosis with and without type 2 diabetes mellitus

Prabhakar Kuruva [a], *Shravika Reddy Kandi* [b], *Subhakar Kandi* [c,*]

[a] *Department of Respiratory Medicine, Government General & Chest Hospital, Osmania Medical College, Hyderabad, India*

[b] *Mallareddy Institute of Medical Sciences, Hyderabad, India*

[c] *Government General & Chest Hospital, Hyderabad, India*

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ABSTRACT

Background: The bidirectional association between tuberculosis (TB) and diabetes mellitus (DM) is currently one of the major concerns for clinicians, as DM affects the disease presentation and clinical outcome of TB and vice versa. The interest in diabetes mellitus and tuberculosis is mounting rapidly and it promises to be an exciting time for researchers involved in the study of dual diseases.

Methods: A prospective case control study was conducted over a period of one year, on patients diagnosed with pulmonary tuberculosis (PTB) with and without associated type 2 diabetes mellitus, who were admitted in a tertiary care hospital. Pulmonary TB patients with diabetes were labelled as the case group, and those without diabetes were labelled as the control group. A total number of 63 patients in the case group were compared with 63 patients in the control group.

Results: In the present study, clinical symptoms were similar in both the case and control groups, except for haemoptysis (27% vs. 12.7%) and weight loss (96.8% vs. 84.1%), which were significantly more predominant in the case group. There was a significant radiological involvement of the lower lung fields (46% vs. 17.5%) with cavitations (42.9% vs. 20.6%) in the case versus the control group. The sputum conversion at the end of the 2nd month was 92.1% in the control group and 55.6% in the case group ($p = 0.001$). In addition, cure rate in the control group was notably higher than in the case group (81% vs. 61.9%). The proportion of treatment failures were more among the case group (14.3%) as compared to the control group (1.6%).

Conclusion: The present study concludes that, diabetes certainly affects the clinical, bacteriological and radiological presentation and treatment outcome of pulmonary tuberculosis.

* *Corresponding author*. Petunia 1, Nectar Gardens, Madhapur, Hyderabad, 500081, India.
E-mail addresses: subhakar_kandi@yahoo.co.in, drsubhakar@gmail.com (S. Kandi).

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1. Introduction

Tuberculosis is the most prevalent infection in the world. India bears one-fourth of the global tuberculosis burden. Despite all national and international efforts to control and eliminate tuberculosis, an estimated 10 million active tuberculosis cases were noted in the year 2019 of which an estimated 1.2 million people died.[1] Based on a compilation of studies from different parts of the world, it has been projected that the maximum increase in diabetes is most likely to occur in India.[2]

Patients with diabetes mellitus are at a higher risk of tuberculosis. This has been highlighted by several retrospective and prospective studies.[3] In view of this dual epidemic, World Health Organization (WHO) and the International Union against Tuberculosis and Lung Disease issued global recommendations for tuberculosis and diabetes in 2011.[4] Studies have noted that the risk of developing TB was 11–18 times greater in than in the normal population.[3] There is a physiologic basis for the increased incidence of pulmonary tuberculosis in diabetics[5,6,.7] Diabetes increases the risk of developing TB disease about threefold, as reported in a systematic review of 13 observational studies. The risk estimates from these individual studies was notably broad ranging from 0.99 to 7.83.[3] The bidirectional association between tuberculosis (TB) and diabetes mellitus (DM) is currently one of the major concerns for clinicians, as DM affects the disease presentation and clinical outcome of TB and vice versa.[8] This co morbidity has been known since the beginning of the 20th century. However, a recent increase in the number of DM patients, attributed mainly to the modern lifestyle changes, created interest in further assessing the association between these two diseases.[9] This co-epidemics is emerging predominantly in resource poor countries where PTB is highly endemic, with an ongoing burden of DM.[10]

Many cases with advanced TB have resulted from a delayed diagnosis of diabetes mellitus. Hence, the WHO is currently recommending bidirectional screening for patients with diabetes and tuberculosis, which will help to identify these diseases early and start appropriate management immediately.

With this background, the present study was undertaken with the objectives of studying the clinical spectrum of pulmonary tuberculosis in patients with diabetes mellitus, as well as the effect of diabetes mellitus on the outcome of patients with pulmonary tuberculosis.

2. Methodology

A prospective case control study was conducted over a period of one year on patients diagnosed with pulmonary tuberculosis admitted in Government General & Chest hospital, Hyderabad, a tertiary care institute for tuberculosis and chest diseases. Patients with pulmonary tuberculosis above the age of 15 years were registered for the study. Among these patients, those with type 2 diabetes mellitus were labelled as the case group, while those without diabetes was labelled as controls.

Patients who gave informed consent and were willing to participate in regular follow up were included in the study. Patients with HIV, those who were on immunosuppressive therapy, individuals with history of drug resistant tuberculosis, patients with evidence of extra pulmonary tuberculosis and those who did not give informed consent were excluded from the study. A total of 63 cases that satisfied inclusion and exclusion criteria were enrolled. On a 1:1 basis, 63 controls were also enrolled.

Data was collected using a structured questionnaire to elicit demographic and clinical variables including history of diabetes in all patients who were diagnosed with pulmonary tuberculosis at Govt General and Chest Hospital, Hyderabad. Written and informed consent was taken from patients before enrolling them into study. The study was approved by the institutional ethics committee and review board of the hospital.

The assessment included patient's demographic profile, clinical features and chest X-ray findings. Laboratory tests such as sputum for acid fast bacilli (AFB), glycosylated haemoglobin (HbA1c), fasting blood sugar, post prandial blood sugar levels were taken into consideration. History of co morbidities, human immunodeficiency virus (HIV) status, history of drug intake and any other significant history was also noted.

All patients with history and clinical features suggestive of pulmonary tuberculosis were subjected to chest X-ray and sputum for AFB with fluorescent staining by auramine-rhodamine stain at the designated microscopy centre at our hospital. Culture and drug susceptibility tests of sputum were performed for retreatment cases to rule out drug resistant tuberculosis.

Based on the results, sputum positive cases as well as sputum negative cases with clinical, radiological and culture positive pulmonary tuberculosis were enrolled for the study. Among sputum negative cases, outcome assessment was done by radiological improvement and culture conversion.

Blood samples were collected from all the cases and controls to measure random, fasting and post lunch blood sugar, as well as HbA1c. Criteria for the diagnosis of DM includes: symptoms of diabetes plus random blood sugar value $\geq$ 11.1 mmol/l (200 mg/dl) or fasting plasma glucose $\geq$ 7.0 mmol/l (126 mg/dl) or 2-hour plasma glucose $\geq$ 11.1 mmol/l (200 mg/dl) during an OGT test.

Patients in the case group, who have met the above criteria, were tested for glycosylated haemoglobin (HbA1c) levels using High Pressure Liquid Chromatography technique. A HbA1c value of 6.5% or above was considered diagnostic of DM, as per American Diabetes Association (ADA).

All the patients were followed up until the completion of treatment according to the guidelines of National Tuberculosis Elimination program (NTEP). This was done to establish the correlation of all factors in deciding treatment outcome. The software used for data analysis was SPSS v21. Categorical data was represented as frequencies and percentages. A chi square test was used to test significance for categorical data. A p-value less than 0.05 was considered as statistically significant.

3. Results

A comparative study of the impact of coexistence of diabetes mellitus and pulmonary tuberculosis on clinical spectrum and management was done in equally distributed (1:1), randomized patient groups.

In the present study, males are more affected with pulmonary tuberculosis than females, both in case (47/63, 74.6%) and control groups (42/63, 66.7%).

It was observed that, patients who are underweight are less in number among the case group (32, 50.80%) compared to the controls (52, 79.40%). Patients who are overweight are more in the case group (7.9%) than the control group (1.6%) (Fig. 1).

In the present study, clinical symptoms were similar in both the case and control groups, except for haemoptysis (27% vs. 12.7%) and weight loss (96.8% vs. 84.1%), which were significantly more predominant in the case group (Table 1). Out of 63 patients, 23 (36.5%) from the case group and 10 (15.9%) from the control group had a family history of diabetes mellitus.

It was observed that 6.3% of cases and 17.5% of controls were negative for sputum AFB at diagnosis and this was statistically significant [Table 2: Chi square = 13.151, P value = 0.011 (S)]

It was observed that sputum conversion at the end of two months of treatment in the control group was 92.1% while in the case group, it was only 55.6% and this distribution of patients was found to be statistically significant. [Table 3: Chi square = 24.822, P value = 0.001 (S)]

In the case group, 14.3% of patients are sputum positive even at the end of treatment, while it was only 1.6% in the control group and these observations are statistically significant [Table 4: Chi square = 9.026, P value = 0.037 (S)]

In our study, 38.10% had bilateral lesions in the case group compared to 17.5% in the control group and this finding was found to be significant. [Table 5: Chi square = 6.686, P value = 0.035 (S)]

It was observed both in the control case groups that, involvement of the upper zone on chest X-ray was common (case group - 93.7% and control group- 96.8%). In contrary to this, involvement of the lower zone was significantly higher in the case group (46% vs. 17.5%) [Table 6: Chi square = 10.585, p value = 0.001 (S)]

In the present study, consolidation was found in 68.30% in the case group and 60.30% in the control group and Infiltrates were observed in 22.20% in the case group and 17.50% of patients in the control group. These observations have no statistical significance.

Cavitary lesions on chest x-ray were found in 42.9% of patients in the case group and 20.6% in the control group which was statistically significant [Table 7: Chi square = 6.190, P value = 0.013].

There was 81% cure rates in the control group compared to 61.9% in the case group which clearly shows that diabetes mellitus significantly impacts treatment outcome of pulmonary tuberculosis. The proportion of treatment failures were more among the case group (14.3%) as compared to the control group (1.6%). Similarly deaths were also more in the case group than control group (14.3% vs. 1.6%). There was a statistically significant difference for all these observations. [Table 8: Chi square = 9.600, P value = 0.048 (S)]

4. Discussion

A total number of 63 tuberculosis patients with diabetes mellitus were compared with 63 tuberculosis patients without diabetes mellitus to analyse the impact of diabetes mellitus on the clinical spectrum and outcome of pulmonary tuberculosis.

In the present study, there were a total number of 47 males (74.6%) and 16 females (25.4%) in pulmonary tuberculosis with diabetes mellitus group (DM-TB) and 42 males (66.7%) and 21 females (33.3%) in the TB without diabetes mellitus group.

Similar findings were reported in previous studies by Ruslami R et al,[11] Singla R et al,[12] Deshmukh PA et al[13] and Tripathy and Kar.[14] The higher incidence of co-morbidity in males could be explained by the fact that both tuberculosis and diabetes are more common in males. Another reason

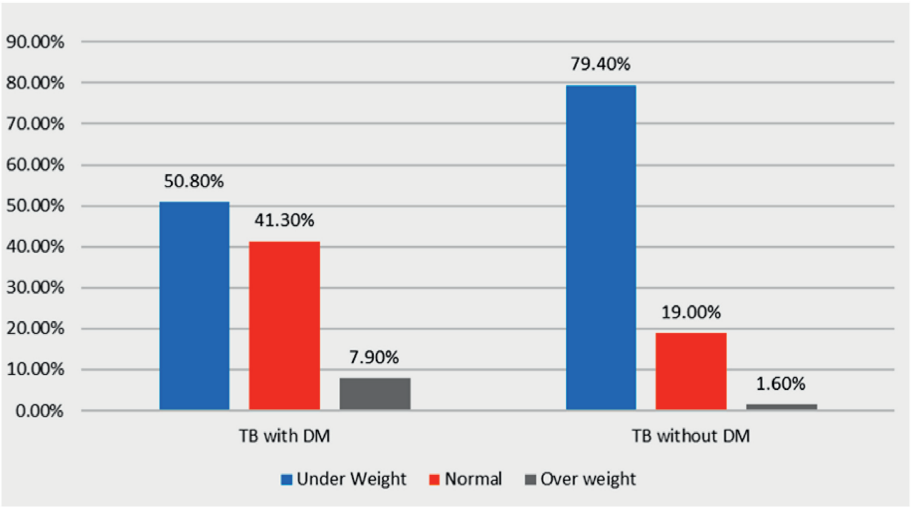

Fig 1 – Case vs. control - distrubution based on weight.

Table 1 – Significance of symptoms at presentation for case vs. control groups.

Symptom	Case Group	Control Group	Total Patients	Chi square value	P value
Cough with sputum	63/63 (100%)	63/63 (100%)	126/126 (100%)	–	–
Fever	62/63 (98.4%)	63/63 (100%)	125/126 (99.2%)	1.008	0.315
Dyspnoea	20/63 (31.7%)	12/63 (19.0%)	32/126 (25.4%)	2.681	0.102
Haemoptysis	17/63 (27.0%)	8/63 (12.7%)	25/126 (19.8%)	4.042	0.044
Chest pain	9/63 (14.3%)	9/63 (14.3%)	18/126 (14.3%)	0.000	1.000
Weight loss	61/63 (96.8%)	53/63 (84.1%)	114/126 (90.5%)	5.895	0.015

Table 2 – Sputum AFB positivity at baseline among case vs. control groups.

Sputum at baseline	Case Group	Control Group	Total
1+	36 (57.1%)	29 (46.0%)	65 (51.6%)
2+	15 (23.8%)	8 (12.7%)	23 (18.3%)
3+	8 (12.7%)	8 (12.7%)	16 (12.7%)
Neg	4 (6.3%)	11 (17.5%)	15 (11.9%)
Scanty	0 (0.0%)	7 (11.1%)	7 (5.6%)
Total	63 (100.0%)	63 (100.0%)	126 (100.0%)

Chi square = 13.151, P value = 0.011 (S).

Table 3 – Sputum AFB status after 2 months of treatment among case vs. control groups.

Sputum after 2 months of treatment	Case Group	Control Group	Total
1+	26 (41.3%)	4 (6.3%)	30 (23.8%)
2+	2 (3.2%)	0 (0.0%)	2 (1.6%)
Neg	35 (55.6%)	58 (92.1%)	93 (73.8%)
Scanty	0 (0.0%)	1 (1.6%)	1 (0.8%)
Total	63 (100.0%)	63 (100.0%)	126 (100.0%)

Chi square = 24.822, P value = 0.001 (S).

Table 4 – Sputum AFB status at the of treatment among case vs. control groups.

Sputum at the end of treatment	Case Group	Control Group	Total
1+	7 (11.1%)	0 (0.0%)	7 (5.6%)
Neg	41 (65.1%)	52 (82.5%)	93 (73.8%)
Scanty	2 (3.2%)	1 (1.6%)	3 (2.4%)
NA	13 (20.6%)	10 (15.9%)	23 (18.3%)
Total	63 (100.0%)	63 (100.0%)	126 (100.0%)

Chi square = 9.026, P value = 0.037 (S).

Table 5 – Localization of chest Xray lesion among case vs. control groups.

Side of the lesion on X ray	Case Group	Control Group	Total
Bilateral	24 (38.10%)	11 (17.50%)	35 (27.8%)
Left	18 (28.60%)	24 (38.10%)	42 (33.3%)
Right	21 (33.30%)	28 (44.40%)	49 (38.9%)
Total	63 (100.0%)	63 (100.0%)	126 (100.0%)

Chi square = 6.686, P value = 0.035 (S).

Table 6 – Lower zone radiological presentation among case vs. control groups.

Lower zone changes	Case Group	Control Group	Total
Present	29 (46.00%)	11 (17.50%)	40 (31.80%)
Absent	34 (54.00%)	52 (82.50%)	86 (68.20%)
Total	63 (100.0%)	63 (100.0%)	126 (100.0%)

Chi square = 10.585, p value = 0.001 (S).

Table 7 – Chest X-ray cavity presentation among case vs. control groups.

Cavity	Case Group	Control Group	Total
Present	27 (42.90%)	13 (20.60%)	40 (31.80%)
Absent	36 (57.10%)	50 (79.40%)	86 (68.20%)
Total	63 (100.0%)	63 (100.0%)	126 (100.0%)

Chi square = 6.190, P value = 0.013.

Table 8 – Treatment outcome distribution among case vs. control groups.

Treatment outcome	Case Group	Control Group	Total
Completed	2 (3.20%)	2 (3.20%)	4 (3.10%)
Cured	39 (61.90%)	51 (81.00%)	90 (71.40%)
Default	6 (9.50%)	6 (9.50%)	12 (9.50%)
Died	7 (11.10%)	3 (4.80%)	10 (8.00%)
Treatment failure	9 (14.30%)	1 (1.60%)	10 (8.00%)
Total	63 (100.0%)	63 (100.0%)	126 (100.0%)

Chi square = 9.600, P value = 0.048 (S).

could be that the number of male patients seeking admission into hospital is more than females.

A higher percentage of underweight patients was noted in the TB without DM group 79.4%), compared to 50.8% TB with DM group. But the percentage of overweight patients was more in the TB with DM (7.9%) arm compared to TB without DM (1.6%). Ruslami R et al[11] study also concurred with our observation. Similarly, Balde NM et al[15] reported 23% of TB-DM patients were obese (BMI > 30 kg/m2), compared with 3% of non-diabetic patients with pulmonary TB.

The predominant symptoms in case and control groups were cough with sputum, fever and weight loss. Similar findings were reported by Goswami et al,[16] Feleke Y et al,[17] Parvaneh Baghaei.[18] Haemoptysis was significantly higher in TB with DM (27%) compared to TB without DM (12.7%). Similar findings were reported by Parvaneh Baghaei et al.[18]

Family history of diabetes mellitus was noted to be higher in case group (36.5%) compared to TB without DM (15.9%). Similar results were seen in Soundararajan Raghuraman et al study.[19] So family history of DM in pulmonary TB patients should be considered as a risk factor and screening for DM in all such patients must be done for early.

Sputum for AFB at end of 2nd month of treatment was positive in 44.5% in TB-DM group, compared to 7.9% in TB without DM group. Similar findings were reported by Guler et al,[20] Tatar et al,[21] Hara et al,[22] Wada et al.[23] This clearly shows that DM significantly delays the sputum conversion in Pulmonary TB patients.

Sputum conversion was seen in 92.1% in control group at the end of 2 months of treatment, compared to a significantly low 55.6% among the DM-TB group. There are studies by Heysell SK et al[24] and Chang JT et al[25] that showed a trend towards increased time to sputum conversion in patients who have associated diabetes mellitus. In a systematic review by Baker et al,[26] 8 out of 9 studies reported delayed sputum culture conversion. This finding may probably be due to improperly controlled blood glucose levels secondary to inadequate anti-diabetic therapy or extensive infection due to high bacterial load. However, contrary to our findings, a study in Indonesia by Alisjahbana B et al showed that, culture conversion proportions were similar in patients with diabetes and patients without diabetes at 2 months (17.1% and 18.3%, respectively).[27]

In the present study, bilateral lung involvement is more common in pulmonary tuberculosis with diabetes mellitus group (38.1%) compared to pulmonary tuberculosis without diabetes mellitus (17.5%). Several studies show bilateral involvement in TB with DM similar to results seen with our study.[28,29,30,31,32] In unilateral involvement, right sided disease was found to be more common in pulmonary tuberculosis. This is predicted to be due to larger lung mass on the right side and straighter course of right bronchus enabling easier entry of air with foreign particles. However, the higher prevalence of bilateral involvement in TB-DM arm can be attributed to rapid bronchial dissemination.

Predominant involvement of the upper zones on chest X-ray was observed in both study groups, non-DM-TB (96.8%) DM-TB group (93.7%). Upper zone involvement is more common in TB because alveolar concentration is more than arterial concentration in upper zones and TB bacilli being aerophilic grow better in the upper zones.

However, the present study shows involvement of lower zone in chest X-ray is more in patients with pulmonary tuberculosis with diabetes mellitus (46%) compared to pulmonary tuberculosis without diabetes mellitus (17.5%). Similar findings were reported in studies by Shaik MA et al[33] and Zuber et al.[31]

Consolidation was more predominant among pulmonary tuberculosis with diabetes mellitus (68.3%) compared to pulmonary tuberculosis without diabetes mellitus (60.3%). Similar findings were observed in studies like Qazi MA et al[29] showing 75/150 (50%) patients in their study with consolidation.

Chest X-ray infiltrates, in addition to consolidation among TB-DM and TB without DM were 22.2% and 17.5% respectively. In the current study, Cavitary lesions in the TB-DM group (42.9%) were more compared to pulmonary tuberculosis without diabetes mellitus (20.6%). Similar findings were reported by Shaik MA et al,[33] M Mani Mala et al[34] and PerezGuzman et al.[35]

Hence patients with cavitations in the lower zone of the lung are associated with a higher incidence to type II diabetes mellitus, and therefore there should be a strong suspicion to identify and treat patients at the earliest.

The current study showed that PTB patients with diabetes mellitus have a lower cure rate (61.9%) compared to non-DM-TB patients (81.0%). Treatment failure in pulmonary tuberculosis with diabetes mellitus was more (14.3%) compared to that of pulmonary tuberculosis without diabetes mellitus (1.6%). It could be due to uncontrolled blood glucose levels, more bacillary load, failure of sputum conversion, and decreased plasma concentration of anti-tuberculous drugs.

The present study also showed more deaths among PTB with DM group (11.1%) compared to the PTB without DM group (4.8%). In a descriptive case–control study by Mboussa and colleagues,[36] treatment failure or death was seen in 41% of the patients with tuberculosis and diabetes mellitus, but in only 13% of those with tuberculosis alone. Similar findings were also reported by several other studies.[27,37–39]

5. Conclusion

The present study concludes that, diabetes mellitus certainly affects the clinical, bacteriological and radiological presentation of pulmonary tuberculosis. Diabetes mellitus significantly impacts even the treatment outcome of pulmonary tuberculosis. Therefore, all patients with pulmonary tuberculosis should be screened for diabetes mellitus. Similarly all high-risk diabetic patients should be screened for Tuberculosis. Early detection and an effective control of DM in patients with Pulmonary Tuberculosis may significantly improve outcome of both.

Author contributions

SK conceptualized and designed the study. PK and SR recruited study subjects and controls, collected patient data and samples, carried out data entry, followed all the subjects and controls till the end of study period with regular reporting to SK. PK and SR were also involved in preparing and finalizing the manuscript with critical suggestions and inputs from SK.

Ethical approval

The study was approved by the Institutional Ethical Committees of Government General & Chest Hospital, Hyderabad (affiliated to Osmania Medical College). Informed written consent was obtained from all study participants.

Conflicts of interest

The authors have none to declare.

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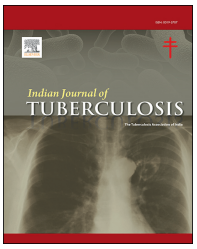

Original Article

Evaluation of the performance of a novel sputum processing ReaSLR methodology for culture of sputum samples in solid and liquid media in comparison with modified Petroff's method☆

Anita Sharma [a], *Ashwini Agarwal* [b,*]

[a] *Department of Microbiology, Military Hospital, Dehradun, India*
[b] *Department of Microbiology, All India Institute of Medical Sciences, Jodhpur, India*

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ABSTRACT

Background: Early case detection by sputum smear microscopy is a crucial step in the control of pulmonary tuberculosis in high burden countries. Due to low sensitivity of this rapid and cost effective method, culture of *Mycobacterium tuberculosis* (MTB) is considered as the gold standard. Modified Petroff's method using 2%–4% sodium hydroxide (NaOH) and N-acetyl- L-cysteine (NALC) to digest and at the same time to decontaminate the specimen is widely used in developing countries prior to culture. This method is considered tedious and cumbersome. A novel ReaSLR (**Rea**Metrix's **S**putum **L**iquefying **R**eagent) methodology has been proposed as a simple and low-cost method for sputum processing. This study was undertaken to evaluate the performance of the ReaSLR method of sputum processing prior to culture in comparison to the modified Petroff's method.

Methods: Early morning sputum samples, collected from suspected TB patients, were divided into two equal halves and processed by two different methods i.e modified Petroff's method and ReaSLR method. After processing with different methods, each sample was inoculated in Lowenstein Jensen (LJ) medium and Mycobacteria growth indicator tube (MGIT). Smears were also prepared from the samples processed with modified Petroff's method and graded according to Centers for Disease Control and Prevention (CDC) grading after microscopic examination. Culture results of both the methods were recorded and analysed using SPSS 20.0 version.

Results: On comparing different methods of sputum processing for culture in solid and liquid media, the rate of contamination in both the media was significantly high with ReaSLR method as compared with modified Petroff's method. Also, the mean time-to-detection of MTB growth in LJ medium was significantly less with modified Petroff's method i.e 30.21 days as compared to ReaSLR method (34.23 days; $p < 0.001$). However, the mean time-to-detection of MTB growth in MGIT was similar with both the methods.

Conclusion: Due to the high contamination rate in solid and liquid culture media, ReaSLR method cannot be considered as an alternative to modified Petroff's method for sputum

☆ **Place of study** - Armed Forces Medical College, Pune 411040, India.

* *Corresponding author*. Department of Microbiology, All India Institute of Medical Sciences, Jodhpur, 342008, India. Tel.: +9595666071.
E-mail address: ash.afmc@hotmail.com (A. Agarwal).

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processing prior to culture. The detection of growth of MTB in LJ media was also earlier with modified Petroff's method than ReaSLR method.

1. Introduction

According to global tuberculosis (TB) report 2019, India is one of the top 20 countries with the highest estimated number of incident TB cases and multidrug resistant TB (MDR-TB) cases.[1] For the early and rapid diagnosis of TB, sputum smear microscopy is being used in the high burden countries. In spite of low cost, rapid method and high specificity, the sensitivity of the sputum smear microscopy is low, that varies from 20 to 80%.[2]

Culture (solid/liquid media), being more sensitive than smear microscopy, provides definitive diagnosis of TB. Culture increases the number of tuberculosis cases found, which are smear-negative.[3] Culture of MTB on solid or liquid media is considered as gold standard for diagnosis of TB. Processing of all the sputum samples is required prior to culture as sputum is contaminated to varying degrees by resident flora of upper respiratory tract. Since mycobacteria are slow growing organisms and require long incubation time, these contaminating organisms can overgrow in culture. Digestion, decontamination and concentration procedures are used in processing sputum samples for smear microscopy and culture on solid or liquid media.

There are many methods for sputum sample processing to enhance the recovery of MTB, but none of them are ideal. There is no method which selectively destroys only contaminating flora without harming mycobacteria while processing the sputum sample. A reasonable compromise is to harm as few mycobacteria as possible while destroying maximum of the contaminating bacteria. Modified Petroff's method using 2%–4% Sodium hydroxide (NaOH) and N-acetyl- L-cysteine (NALC) is widely used in developing countries for sputum processing before culture. However, overall processing of sputum samples with modified Petroff's method is cumbersome, tedious and requires special equipments.

A novel ReaSLR (**Rea**Metrix's **S**putum **L**iquefying **R**eagent) methodology for sputum processing has been described as an easy and better alternative to conventional methods for sputum processing.[4] The ReaSLR kit (M/S ReaMetrix, Bangalore, India) contains a ready to use tablet and a 10 ml syringe fitted with ReaFilter (Figs. 1 and 2). The tablet is used to liquefy the pulmonary sample as well as decontaminate other bacteria except MTB. The role of ReaFilter is to remove the debris and other unwanted particulates present in the sputum sample.

According to a study, carried out by Verma S et al in 2013, the positivity rate of ReaSLR method in smear microscopy is higher than that of the modified Petroff's method.[4] There is no published data on utility of this method for decontamination of sputum samples prior to culture. This study was undertaken to evaluate the performance of the ReaSLR method of

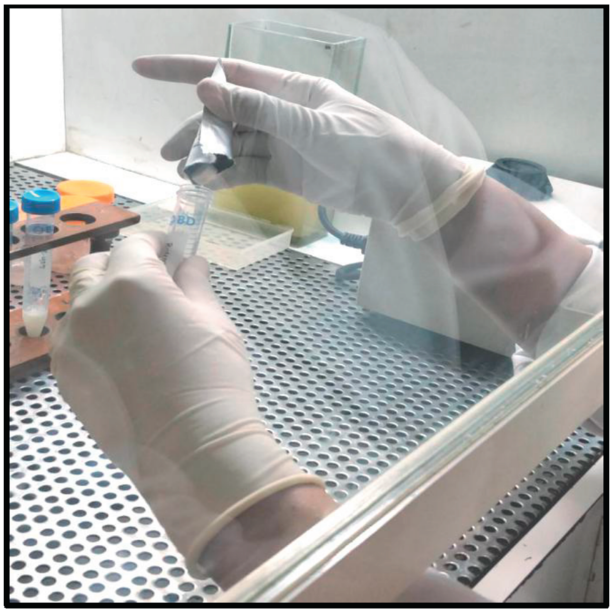


Fig. 1 – ReaSLR tablet added to 2ml of sputum sample.

Fig. 2 – Liquified sputum filtered through ReaFilter Syringe.

sputum processing for culture in solid and liquid media in comparison to the modified Petroff's method.

2. Methods

The prospective study was conducted over a period of 18 months in the Department of Microbiology of a tertiary care teaching college and hospital. The study proposal was approved by Hospital Ethics Committee. Early morning Sputum samples from consecutive patients (age ≥ 18 years) with suspected pulmonary tuberculosis presenting were included in the study after obtaining individual informed consent. Patients with history of taking antitubercular drugs at least for one month were excluded from the study. At least 4ml of sputum sample (mucoid or mucopurulent, not saliva) was included in the study. All biosafety precautions were followed as per recommended guidelines for mycobacterial work.[5]

Sample size was calculated taking sensitivity of modified Petroff's method and ReaSLR method as 40.47% and 90.47% respectively with 95% confidence interval and 5% error of margin.[4] A total of 500 sputum samples were proposed to be included in the study, taking culture positivity rate 25–28% in our setting.

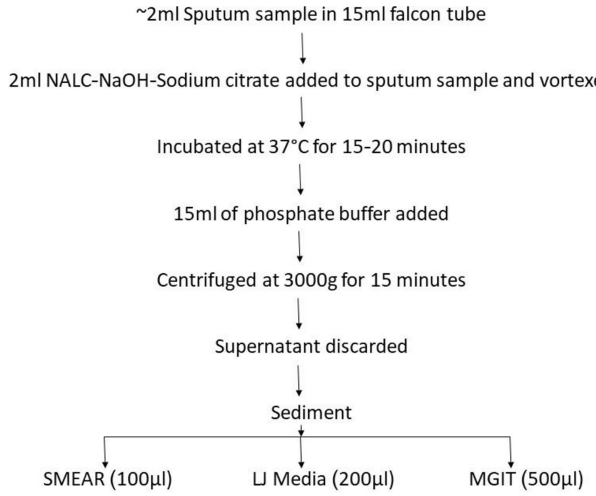
2.1. *Decontamination, digestion and concentration of sputum samples*

Each sputum sample was vortex mixed and transferred into two sterile falcon tubes, at least 2 ml in each tube. Sputum in one tube was processed by Modified Petroff's Method and sputum in other tube was processed by ReaSLR method.

1) **Modified Petroff's Method (NALC-NaOH method)**

2.1.1. *Smear*

Smears were prepared from specimens processed by modified Petroff's method after inoculation into medium using 200μl of re-suspended pellet. After air drying and heat fixing, smears were stained by Ziehl Neelsen (ZN) stain and graded as recommended by Centres for Disease Control and Prevention (CDC).



2.2. *Culture (solid and liquid media)*

2.2.1. *LJ medium inoculation*

200 μl of re-suspended pellet was inoculated on LJ medium slant and incubated at 37 °C after proper labeling with the sample number and date of inoculation. Inoculated slants were inspected after first 48 hrs to rule out bacterial contamination and then examined weekly. In case of any growth on slant, cultures were confirmed for presence of AFB by ZN stain. Cultures were discarded if there was no growth by 8 weeks.

2.2.2. *Manual MGIT inoculation*

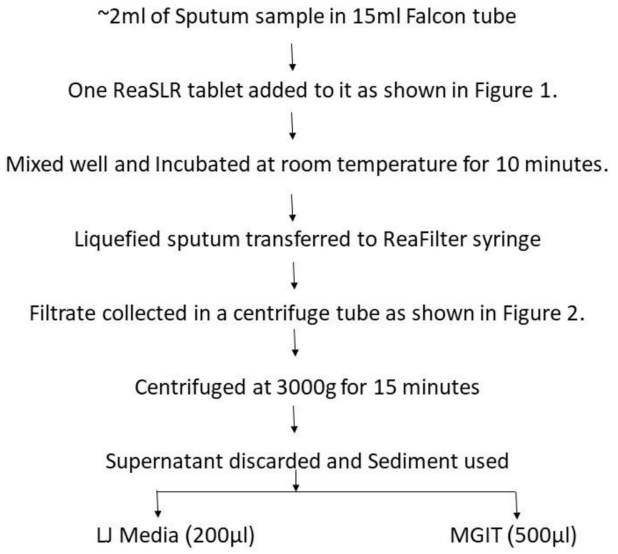
500 μl of re-suspended pellet was inoculated in manual MGIT tubes (Becton–Dickinson, Sparks, MD) as per manufacturer's instructions.[6] Inoculated tubes were incubated at 37 °C and read weekly, along with LJ media, using Micro MGIT fluorescence reader till they became positive or for a maximum of six weeks. On the day of detection, all positive MGIT tubes were examined by ZN staining to confirm the presence of AFB.

2.3. *Identification of MTB complex*

The AFB positive culture was reported to contain 'MTB complex' if positive for presumptive cord formation in liquid media and MPT 64 Antigen (TB Ag MPT64 rapid kit, Standard Diagnostics, Inc., Korea). If both or either test was negative, the culture was reported to contain 'NTM'.

2) **ReaSLR METHOD**

Culture inoculation and then reading of LJ media/MGIT and identification of 'MTB complex' was done **in the similar way as done with modified Petroff's method.**



2.4. Statistical analysis

Data was analysed by using SPSS 20.0 version. Any difference was considered statistically significant if p value was <0.05.

3. Results

Of 531 sputum samples, collected from clinically suspected patients of tuberculosis, on microscopy of smears prepared after processing with modified Petroff's method showed 118 (22.22%) smear positive and 413 (77.78%) smear negative sputum samples.

3.1. Culture results of LJ media and manual MGIT with different methodology

3.1.1. Modified Petroff's method

Of the 531 samples, processed with modified Petroff's method, 22 samples were contaminated in both solid and liquid media and results of these 22 samples were inconclusive. Of the remaining 509 samples, a total of 159 (31.24%) samples were positive in either solid/liquid culture or both. MGIT liquid culture was positive in 147 samples while LJ solid culture was positive in 137 samples. 350 (68.76%) samples were culture negative in both the media.

Of the total 159 culture positive samples, 142 (89.31%) isolates were MTB complex, while 17 (10.69%) isolates were Non Tuberculous Mycobacteria (NTM) as shown in Table 1.

3.1.2. ReaSLR method

Of 531 samples, processed with ReaSLR method, 83 samples were contaminated in both solid and liquid media and culture results of these 83 samples were inconclusive. Of the remaining 448 samples, a total of 117 (26.11%) samples were positive in either solid/liquid culture or both. MGIT liquid culture was positive in 93 samples while LJ solid culture was positive in 115 samples.

Of the total 117 culture positive samples, 105 (89.74%) isolates were MTB complex, while 17 (10.26%) isolates were Non Tuberculous Mycobacteria (NTM) as shown in Table 2.

3.2. Comparison of rate of contamination in solid and liquid media

Of 531 samples processed with modified Petroff's method, there were 29 contaminated LJ media and 43 contaminated MGIT liquid culture. While there were 83 contaminated LJ media and 132 contaminated MGIT liquid culture with ReaSLR method. There were 22 samples, processed with modified Petroff's method, and 83 samples, processed with ReaSLR method, in which both the media (solid/liquid) got contaminated.

Table 1 – Culture results of LJ media and manual MGIT with modified Petroff's method (n = 509).

Isolates	LJ only	MGIT and LJ	MGIT only	Total
MTB complex	11	109	22	142
NTM	01	16	0	17
Total	12	125	22	159

Table 2 – Culture results of LJ media and manual MGIT with ReaSLR Method (n = 448).

Isolates	LJ only	MGIT and LJ	MGIT only	Total
MTB complex	22	81	02	105
NTM	02	10	0	12
Total	24	91	02	117

The rate of contamination in LJ media was significantly higher with ReaSLR method (15.63%) as compared to modified Petroff's method (5.46%) ($p < 0.0001$). Similarly the rate of contamination in MGIT was also significantly higher in ReaSLR method (24.86%) as compared to modified Petroff's method (8.10%) ($p < 0.0001$) as shown in Table 3.

3.3. Comparison of mean time-to-detection of growth (MTB complex) in LJ media

The mean time-to-detection of culture positivity with modified Petroff's method for MTB complex in LJ medium was 30.21 days, while with ReaSLR method it was 34.23 days ($p < 0.001$) as shown in Table 4.

3.4. Comparison of mean time-to-detection of growth (MTB complex) in MGIT

The mean time-to-detection of culture positivity with modified Petroff's method and ReaSLR method in manual MGIT was similar i.e 17.58 days and 17.54 days respectively ($p = 0.9$) as shown in Table 5.

4. Discussion

In spite of high specificity of microscopic examination of sputum specimen, the sensitivity of the smear microscopy is very low and further, it cannot differentiate between *M. tuberculosis* from Non tuberculous mycobacteria, viable from nonviable organisms and cannot ascertain drug-susceptibility status. Due to these limitations, sample processing for recovery of the bacilli from the sputum sample is of paramount importance.

The disadvantages of modified Petroff's method, the most widely used method for sputum sample processing, include

Table 3 – Rate of contamination of solid/liquid media with ReaSLR method and modified Petroff's method (n = 531).

Method	No. of contaminated LJ media (%)	No. of contaminated MGIT* (%)
ReaSLR Method	83/531 (15.63%)	132/531 (24.86%)
Modified Petroff's Method	29/531 (5.46%)	43/531 (8.10%)

Chi Square = 29.10, p-value<0.0001; Chi Square* = 54.19, p-value*<0.0001.

Table 4 – Mean time-to-detection of growth (MTB complex) in LJ Media.

Method	No. of samples	Mean Time-to-detection	SD	t	p
ReaSLR Method	103	34.23	7.01	4.451	<0.001
Modified Petroff's Method	120	30.21	6.47		

Table 5 – Mean time-to-detection of growth (MTB complex) in MGIT.

Method	No. of samples	Mean Time-to-detection	SD	t	p
ReaSLR Method	87	17.54	4.63	0.06	0.9
Modified Petroff's Method	131	17.58	5.16		

the fact that NALC loses its activity rapidly in solution, so digestant should be made freshly daily. Again, the suggested specimen exposure time must be strictly adhered to and the reagents such as NALC are expensive. The step of concentration requires centrifugation at x3000*g* which adds on to the cost of processing. A novel sputum processing ReaSLR method for smear microscopy has been proposed as better alternative to conventional sputum processing methods.[4] The study was carried out to evaluate the performance of ReaSLR method for digestion and decontamination of sputum samples prior to culture. In our study, all 531 sputum samples processed with ReaSLR method were inoculated into one solid culture (LJ medium) and one liquid culture (MGIT).

With modified Petroff's method 142 MTB isolates were identified in culture while with ReaSLR method 105 MTB isolates were identified in culture. ReaSLR method missed 37 MTB isolates which were identified with modified Petroff's method. This could be due to the high rate of contamination in cultures with ReaSLR method. In our study, the contamination rates with ReaSLR method were 15.63% and 24.86% respectively in LJ media and MGIT. While contamination rate with modified Petroff's method was 5.46% for LJ media and 8.10% for MGIT. The contamination rate for modified Petroff's method in our study is similar to that reported in literature.[7,8] Pfyffer et al, Chaudhary et al, and Bunger et al reported contamination rate in LJ media as 8% with modified Petroff's method.[9,10,11] Bunger et al reported 6% rate of contamination with modified Petroff's method in liquid media (MGIT).[11]

The rate of contamination with ReaSLR method was significantly high in both LJ media and MGIT as compared to modified Petroff's method ($p < 0.001$). High contamination rate with ReaSLR method could be due to the ineffective decontamination with chaotropic tablets of ReaSLR kits. Also when the sputum sample was pushed through the ReaFilter syringe, some other bacteria may have passed through the filter along with mycobacteria which contaminated the media.

In the present study, we observed mean time-to-detection of MTB with modified Petroff's method in LJ medium and MGIT as 30.21 days and 17.58 days. Negi et al reported the mean time-to- detection of MTB in LJ media and MGIT 24.3 days and 12.89 days respectively with modified Petroff's method.[12] Pfyffer et al reported the mean time-to-detection of MTB in LJ media and MGIT 23.1 days and 14 days respectively.[9] In comparison, the mean time-to-detection in LJ medium and MGIT with ReaSLR method were 34.23 and 17.54 days respectively. On comparing mean time-to-detection of growth in solid/liquid culture, we observed that there was early detection of MTB in LJ medium with modified Petroff's method as compared to ReaSLR method ($p < 0.001$). While there was no difference in time-to-detection in MGIT with the above two methods ($p = 0.9$). Early detection of growth in LJ media with modified Petroff's method could be due to better concentration of MTB obtained by modified Petroff's method as compared to ReaSLR method.

In our study, there were 8 samples reported as smear positive with modified Petroff's method but there was no growth with both the methods in either solid or liquid media. Smear-positive and culture-negative results could be due to cross-contamination of smears, use of water and stains contaminated with acid fast organisms, and laboratory errors.[13] Also patients are often transferred to our centre after starting anti tuberculosis treatment (ATT) on the basis of clinical or radiological features. This could have also resulted in smear positive and culture negative results.

In our study we found that ReaSLR method did not provide any advantage over modified Petroff's method for culture. ReaSLR method can't be recommended for culture of mycobacteria due to high contamination rates obtained in both solid and liquid media and low culture positivity. We did not find any benefits of the ReaSLR method and do not recommend it as an alternative to conventional methods for sputum processing prior to culture in LJ media and MGIT.

5. Conclusion

The ReaSLR method is not compatible for processing sputum samples prior to culture in solid and liquid media as rate of contamination was higher with ReaSLR method in both solid and liquid media as compared to modified Petroff's method.

Conflicts of interest

The authors have none to declare.

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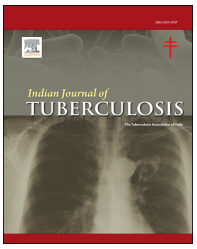

Original Article

Utility of Bronchiectasis severity index (BSI) as prognostic tool in patients with post tubercular bronchiectasis: An experience from a tertiary care hospital in North India

*Amey Deshmukh, Rohit Vadala, Deepak Talwar**

Metro Center for Respiratory Diseases (MCRD), Metro Multispeciality Hospital, Noida, 201301, India

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ABSTRACT

Background: Bronchiectasis severity Index (BSI) score which predicts the severity of the disease along with future exacerbations and mortality rate has been well validated in European patients; however there is paucity of data evaluating its validity in Indian patients. The authors therefore decided to evaluate the utility of BSI to predict exacerbations and mortality rate in patients with post tubercular bronchiectasis presenting to our facility.
Methods: The study was a retrospective observational study done in patients with bronchiectasis secondary to tuberculosis. These patients were followed up for 4 years. BSI was calculated from different variables and descriptive statistics along with regression analysis were used to evaluate utility of BSI.
Results: A total of 48 patients of post tubercular bronchiectasis were included in the study. Majority of our patients belonged to severe bronchiectasis group seen in 23 patients (48%) while those with mild and moderate bronchiectasis were seen in 13 (27%) and 12 (25%) patients respectively. The exacerbation rate in mild group was comparable to the predicted BSI exacerbation at 1 year while the predicted and observed rates were statistically significant for moderate and severe bronchiectasis group (p value < 0.05). Mortality rates at 1 year were comparable in all the groups of bronchiectasis while it was comparable only in mild and moderate group bronchiectasis at 4 years.
Conclusion: Bronchiectasis severity index seems to predict mortality at 1 year in post tuberculosis bronchiectasis. However, it under predicts 1 year and 4 year exacerbation rates. Hence BSI may not be useful as a prognostic tool in Indian patients with bronchiectasis. Larger multi-centred studies may be required to further evaluate the clinical utility of BSI among Indian population.

* *Corresponding author*. Metro Centre for Respiratory Diseases (MCRD), Metro Multispeciality Hospital, Sector 11, Noida, 201301, India. 9899050000(mobile).
E-mail address: dtlung@gmail.com (D. Talwar).
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1. Introduction

Bronchiectasis is defined as chronic respiratory disease characterized by abnormal and irreversible dilatation of the airways, associated with compromised host defences, inflammation, prolonged colonization with bacteria and recurrent respiratory infections.[1] Bronchiectasis is usually associated with chronic sputum production, multiple exacerbations and dyspnoea, which worsens quality of life gradually.[2] The deterioration in lung function negatively impacts the number of exacerbations, hospitalizations, health costs and mortality.[3]

Exacerbation of bronchiectasis is defined as an acute deterioration with worsening local symptoms (cough, increased sputum volume or change of viscosity, increased sputum purulence with or without increasing wheeze, breathlessness and haemoptysis) along with systemic manifestation.[4]

The Indian registry on Bronchiectasis has shown that there is increasing burden of this neglected chronic lung disease. Post-tubercular bronchiectasis is the commonest cause of non-cystic bronchiectasis in India.[5] The severity of bronchiectasis is one of the important factors determining the outcome of patient of bronchiectasis. There are different scoring systems to grade the severity however, bronchiectasis severity index (BSI) scoring is the most commonly used prognostic tool in the western world.

BSI has been well validated in European patients; however there is lack of data evaluating its validity in Indian patients. BSI, in addition to predicting mortality rates also provides additional information on prediction of frequency of exacerbation and hospitalization. As most of the bronchiectasis patients in India are secondary to tuberculosis, the authors in current study aimed to evaluate and explore the utility of BSI as a prognostic tool in predicting the mortality and exacerbation rates these patients presenting to the tertiary care hospital in North India.

2. Materials and methods

The present study is retrospective observational study conducted at metro centre for respiratory diseases (MCRD), Noida. Patients with clinically and radiological evidence of bronchiectasis were included from medical records department (MRD). Patients with age more than 18 years with bronchiectasis were enrolled. Patients with past history of tuberculosis were included and those with secondary to cystic fibrosis, interstitial lung disease and active pulmonary tuberculosis with mycobacterium tuberculosis along with atypical mycobacterial infections were excluded.

BSI was calculated from following variables: Age, body mass index (BMI), forced expiratory volume in 1 second (FEV_1), previous hospitalizations in last 2 years, exacerbations in last year, Medical Research Council Dyspnea Scale (MRC), colonization status and radiological extension in high resolution computed tomography (HRCT) scan were collected at baseline along with follow-up visit data on exacerbations and mortality at 1 year and 4 years.

Forced expiratory volume in 1 second (FEV_1) was one of the important pulmonary function test variable of BSI which was recorded in the study. Points were allotted to these individual variables and the BSI score was calculated from the total points of each variable. These patients were then categorised into 3 groups; mild, moderate and severe groups based on their scores of 0–4, 5–8 & 9 and above point. Predicted and observed exacerbations and mortality rates were compared in patients of mild, moderate and severe BSI groups. Chi square test and binary logistic regression analysis were used to study the association of mortality and exacerbations with components of BSI. The study was approved by the institutional ethics committee.

3. Results

A total of 48 clinically and radiologically diagnosed bronchiectasis patients secondary to tuberculosis were included in the study. There were 35 males (73.9%) and 13 females (27.1%). The mean age of patients in our study was 59.37 years (±12.97). Mean BMI of these patients were 22.06mg/kg^2 (±3.66) with majority of the patients (n-38) 79.2% having a BMI of more than 18.5kg/m^2. The mean FEV_1 of our patients was 36.79% (±17.22) and almost 80% of patients had FEV_1 of less than 50% predicted. Mean hospital admissions in the preceding 2 years were 3.53 (±1.6) with mean exacerbations of 1.7 (±0.8) in past 1 year. Most of the patients (62.5%) had a MRC dyspnea scale of 1–3 with a mean MRC dyspnea scale of 3.08 (±0.9).

BSI was calculated from its components which were age, BMI, FEV_1, previous hospitalizations in last 2 years, exacerbations in last year, Medical Research Council Dyspnea Scale (MRC), colonization status and radiological extension in HRCT scan. Colonisation with pseudomonas and other microorganisms (*Klebsiella pneumoniae* followed by *Escherichia coli*, and Acinetobacter species) were seen 12 patients (25%) and 8 patients (17%) respectively. Bronchiectasis was predominantly tubular seen in 35 patients (73%) followed by cystic and varicose in 16% and 11% patients respectively. Radiological extension of bronchiectasis was noted in HRCT with mean involvement of 2.21 (±0.6) lobes.

The patients were characterised into mild, moderate and severe bronchiectasis based on BSI score of 0–4, 5–8 and more than 9 points. Majority of our patients belonged to severe bronchiectasis group seen in 23 patients (48%). Patients with mild and moderate bronchiectasis were seen in 13 (27%) and 12 (25%) patients respectively.

The comparison of exacerbation at 1 year amongst predicted and observed showed that there was significant statistical difference noted for moderate and severe bronchiectasis group. The exacerbation in mild group was comparable to the predicted BSI exacerbation.

The comparison of exacerbation rates at 4 year observed to the predicted showed statistical significance only in severe group, while in mild and moderate group both observed and predicted rates were comparable.

The comparison of the observed mortality rates at 1 year to the predicted showed no statistical significance. However, comparison of observed mortality rates at 4 year to the predicted showed statistical significance only in the severe group. The predicted mortality rates at 4 year were comparable to the observed rates amongst mild and moderate

group. There was no significant association of mortality and exacerbations with components of BSI scoring system using regression analysis.

4. Discussion

Bronchiectasis is a heterogeneous and neglected chronic respiratory disease which leads to increased morbidity and mortality. Hence severity assessment and stratification of this multidimensional disease is of utmost importance in the management. Although no single parameter has been used to determine the severity and prognosis of the disease, few scoring systems have been developed for the same.

Recently an Indian registry on bronchiectasis has been established to describe the characteristics of bronchiectasis patients in India. Due to high disease burden of tuberculosis, there has been increasing number of post TB sequelae like bronchiectasis, fibrosis and chronic obstructive pulmonary disease. Post tubercular bronchiectasis constitutes an important and a major cause for bronchiectasis in India.[5]

Bronchiectasis severity index (BSI) is commonly used to stratify patients with non-cystic fibrosis bronchiectasis and has been validated as a prognostic tool in European patients. There is however a limited data on its usefulness in Indian patients. BSI scoring includes age, BMI, FEV_1, previous hospitalization, exacerbation frequency, Medical Research Council Dyspnea Scale (MRC), colonization status and radiological appearance.[6]

Our study population had patients with a mean age of 59.37 (±12.97) years which were similar with the studies by Hikmet Coban et al[7] and Minov et al[8] had mean age of 53 (±15.1) and 63.4 (±8.1) years respectively.

Majority of our study patients belonged to the normal weight band (BMI >18.5kg/m^2) while about 20% of patients were underweight (BMI <18.5kg/m^2). The study by Minov et al[8] had mean BMI of 24.3kg/m^2 (±3.7), while it was similar to our study which was 22.06 kg/m^2 (±3.66). Chalmers et al[9] reported that only about 6.9% patients of their study population were underweight while it was higher in our study.

Lung function is one of the important determinants of severity of bronchiectasis. Patients were categorised based on different severity grades of FEV_1. Majority of the patients, 20 (41.6%) had FEV_1 of 30–49% and only one patient (2%) had FEV_1 of >80%. Mean FEV_1 amongst all patients was 36.79% (±17.22). A study by Chalmers et al[9] showed that around 37% of patients with bronchiectasis had FEV_1 >80% while our study population had only (1.6%) patients. The patients with <30% FEV_1 were seen in only 3.7% patients in Chalmers' study while our study population it was seen in 40.3% patients. Other studies by Hikmet Coban et al[7] and Minov et al[8] reported higher mean FEV_1 55.5% (±21.2) and 57.6% (±8.7) respectively. The study by M.R. Loebinger et al[10] had mean FEV_1 of 65.8% (±28.1).

Past hospitalisations and exacerbations did constitute one of the important variables of BSI. In a study by Chalmers et al,[9] 21.8% patients had past hospitalization due to respiratory problems in last 2 years while in our study it was reported in 37.5% cases. In our study, there were 20 (41.6%) patients who had two or more exacerbations in their past year. The mean exacerbation in past 1 year noted in our study was 1.7 (±0.8). The study by Minov et al[8] had a higher mean past exacerbations of 2.12 (±0.54) in the past 1 year.

The majority of patients 19 patients (39.5%) in our study had a MRC dyspnoea score of grade 3 while (37.5%) of patients in study by Chalmers et al[9] had MRC dyspnoea score of grade 1. The patients in the study by Minov et al[8] had mean MRC dyspnoea score of 1.83 (±0.63), while our study showed a mean MRC dyspnoea score of 3.08 (±0.98). Mean lobe involvement in HRCT was 2.21 (±0.68) in our study was similar to that by Minov et al[8] study which had mean lobes involvement of 2.25 (±0.78). Colonization rates with pseudomonas aeruginosa was noted in 12 patients (25%) which were higher when compared to the results by Chalmers et al[9] study seen in 11.5% patients, Minov et al[8] study in 8.1% patients and Hikmet Coban et al[7] study in 9.4% patients.

The predicted exacerbations at 1 year and the observed exacerbations were compared with different severity grades according to the BSI scoring. There was a significant statistical difference noted between the two in moderate and severe bronchiectasis group while there was no difference between observed and predicted exacerbations in the mild group. Coban et al[7] study found significant association between predicted and observed exacerbation among all the severity grades of bronchiectasis.

The predicted exacerbations at 4 year and the observed exacerbations were also compared with different severity grades according to BSI scoring. There was a significant statistical difference noted between the two in the severe bronchiectasis group. However there was no difference noted between observed and predicted in the mild and moderate group. Bronchiectasis severity index score is a good predictor of exacerbation at 1 year and 4 year for all BSI categories.[11] A multi-centric study in European population showed association between BSI severity category and exacerbation.[12]

The comparison of observed and predicted mortality at 1 year and 4 year showed no statistical significance and the predicted rates were comparable with observed mortality rates. Several studies confirmed that BSI was a good predictor of mortality in European patients. The study by Ellis et al[13] and Loebinger et al[10] showed that BSI score was a good predictor of mortality at 1 year. Saleh and Hurst[14] in their study confirmed that BSI score predicted long-term mortality in all 3 categories of BSI.

In our study, when we studied association of mortality and exacerbations with components of BSI scoring system, no statistical significance seen with any component of BSI scoring system. In study by Chalmers et al,[6] association of mortality and exacerbations with components of BSI scoring system showed statistical significance with all components of BSI scoring system.

The profile of Indian patients was found to be different from their western counter parts suggesting a possibility of a new phenotype of patients with bronchiectasis. Indian patients were younger, underweight, had a poorer lung function, and were more severely dyspnoeic with severe disease suggested by higher BSI and a higher colonisation rates by *Pseudomonas aeruginosa*. Possible reasons for such a finding would be due to poor socio-economic status, higher illiteracy rate and tuberculosis burden, poor oral and bronchial hygiene and lack of proper health care facility, irrational antibiotic usage

Table 1 – Showing comparison between observed and predicted exacerbations and mortality rates among different severity grades of bronchiectasis.

Exacerbation	1yr			4 yr		
	Observed %	Predicted (as per BSI)%	P value	Observed %	Predicted (as per BSI)%	P value
Mild (0–4)	11.8	0–3.4	0.939	47.0	0–9.2	0.313
Moderate (5–9)	52.9	1–7.2	**0.02**	58.8	9.9–19.4	0.514
Severe (9 & above)	78.8	16.7–52.6	**0.006**	92.9	41.2–80.4	**<0.001**
Mortality	**1yr**			**4 yr**		
	Observed %	Predicted (as per BSI)%	P value	Observed %	Predicted (as per BSI)%	P value
Mild (0–4)	5.8	0–2.8	0.57	5.8	0–5.3	0.195
Moderate (5–9)	5.8	0.8–4.8	0.24	17.6	4–11.3	0.108
Severe (9 & above)	32.1	7.6–10.5	0.302	39.2	9.9–29.3	**0.04**

(*$p < 0.05$, statistically significant).

and supervised physiotherapy training for patients with bronchiectasis in India. Our patients were more prone for exacerbations along with higher mortality. The findings of our study were similar to the Indian bronchiectasis registry which is a multi-centred cross-sectional data of bronchiectasis patient.[15]

Limitation of our study was that it was a single centre experience from a small group of patients. Since the numbers of non-TB bronchiectasis patients were low we did not look into the differences between BSI amongst different aetiologies of bronchiectasis and more prospective studies are needed to yield better results.

5. Conclusion

Bronchiectasis severity index seems to predict mortality at 1 year in post tuberculosis bronchiectasis. However, it under predicts exacerbation at 1 year in moderate and severe bronchiectasis patients. It also under predicts 4 year exacerbations and mortality in severe bronchiectasis patients. Hence BSI may not be used as a prognostic tool in Indian patients with bronchiectasis. Ongoing data collection into Indian registry and long term multi-centred studies may be required to further evaluate clinical utility of BSI among Indian patients.(see Table 1)

Author contributions

Amey Deshmukh: Literature search, Clinical studies, Experimental studies, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review; **Rohit Vadala**: Concepts, Design, Definition of intellectual content, Literature search, Clinical studies, Experimental studies, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review; **Deepak Talwar**: Concepts, Design, Definition of intellectual content, Literature search, Clinical studies, Experimental studies, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review, Guarantor

Conflicts of interest

The authors have none to declare

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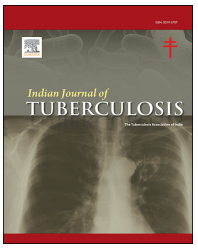

Original Article

Noncompliance of treatment among tuberculosis patients in intensive phase at Kalutara District of Sri Lanka

Nithin Ranawaka [a,*], *Sumal Nandasena* [b], *Sunil De Alwis* [c]

[a] *Postgraduate Institute of Medicine, University of Colombo, Sri Lanka*
[b] *Regional Director of Health Services, Kalutara District, Sri Lanka*
[c] *Ministry of Health, Nutrition and Indigenous Medicine, Sri Lanka*

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ABSTRACT

Background: Tuberculosis (TB) is an ancient disease and remains to be a public health problem all over the world. Noncompliance of treatment among TB patients affect the control of disease, leading to increased burden of the disease, mortality, drug resistant and relapse. Assessing the factors associated with noncompliance of TB treatment will be useful to reduce the noncompliance and burden.

Objectives: To assess the factors associated with noncompliance of treatment among TB patients in intensive phase at Kalutara District, Sri Lanka.

Methods: A descriptive cross-sectional study was conducted among the new TB patients registered at District Chest Clinic (DCC), Kalutara for a period of six months. A questionnaire was administered for total study population registered during the data collection period. The relevant data were abstracted from registers and records maintaining at the DCC.

Results: Data were collected from 252 patients [males = 160 (63.5%) and females = 92 (36.5%)]. The percentage of noncompliance was 18.3% (n = 46) among newly diagnosed TB patients. Only 13.5% (n = 34) of TB patients visited Directly Observed Treatment, short-course (DOTS) provider daily. Majority (61.9%, n = 156) of DOTS providers did not observe for drug intake. The factors significantly associated with noncompliance for TB treatment were (1) not observing the drug intake by DOTS providers, (2) side effects of the drugs, (3) educational level, (4) living environment and (5) absent of a care giver.

Conclusions: Noncompliance of treatment is still a common problem among TB patients. Special emphasis should be made on TB patients based on the factors associated with the noncompliance of the treatment. DOTS providers should adhere to DOTS policy.

* *Corresponding author*. No 123, Andadola, Wathugedara, Sri Lanka.
E-mail address: ranawakanithin@yahoo.com (N. Ranawaka).

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1. Background

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* or occasionally by *Mycobacterium bovis* and *Mycobacterium africanum*. TB commonly affects the lungs, but can affect any other organ in the body.[1]

TB infection can be found in all countries in the world. India, China, Indonesia, Philippines, Pakistan, Nigeria, Bangladesh, South Africa are high burden countries which accounted two third of global burden.[2] In fact, TB is one of the top 10 causes of deaths worldwide. In 2018, 10 million TB cases reported, and 1.5 million died. In 2018, an estimated 1.1 million children became ill with TB and 205 000 children died. Multidrug-resistant TB (MDR-TB) remains a public health crisis and a health security threat. In 2018, World Health Organization (WHO) estimates that there were 484000 new cases with resistance to rifampicin, the most effective first-line drug for TB.[3]

TB remains to be a major public health problem in Sri Lanka. There were 8886 and 8511 people were diagnosed and treated in Sri Lanka for TB in year 2016 and 2017, respectively. Out of them, 5807 and 5785 patients had pulmonary TB (PTB) in year 2016 and 2017, respectively. Kalutara district accounts for more than 6% of total cases reported in Sri Lanka, the third highest TB prevalent district. In year 2017, 568 TB patients were reported from district of Kalutara.[4]

National Programme for Tuberculosis Control and Chest Disease (NPTCCD) is the central level organization responsible for TB control activities in Sri Lanka. NPTCCD network consisted of 26 District Chest Clinics (DCC), chest wards and National Tuberculosis Reference Laboratory. Under each DCC, there are several DOTS centers to serve patients. DCC, itself can serve as DOTS center.

Compliance to the TB treatment is essential to cure the disease, prevent drug resistance to the TB treatment and minimize the transmission of bacilli.[5] The DOTS centers assure the compliance of the drug treatment of TB patients.

There are two categories of treatment regime for TB. Category one treatment regime included all newly diagnosed TB cases whereas category two treatment regime consisted with all retreatment cases.[1] Category one treatment regime consisted of two phases, first phase is intensive phase and second phase is continuation phase. In intensive phase Isoniazid, Rifampicin, Pyrazinamide, Ethambutol are used daily for two months. All the patients should give DOTS on daily basis during the intensive phase as it is the most important period for preventing antibiotic resistant.[1] In continuation phase Isoniazid, Rifampicin are used daily for four-months.[1] DOTS strategy is not compulsory in continuation phase. Thus, the factors associated with noncompliance in "intensive phase" and "continuation phase" could be different and required to assessed separately. The present study aimed to identify sociodemographic factors and health care service-related factors associated with noncompliance to TB treatment in intensive phase.

During the intensive phase of TB, DOTS strategy was adapted by the WHO to minimize the noncompliance. The DOTS strategy adapted in 1997 in Sri Lanka.[1] With DOTS, the patient not bear the sole responsibility for compliance of the treatment, but also health care worker, government and community services should provide range of supports for TB patient to continue and finish treatment successfully.[6] DOTS provider has a major role in DOTS strategy. Their duties include[1] observe patient drug intake and swallowing daily during intensive phase,[2] provide health education to the patients,[3] refer patients to chest clinic if there is an any side effect of the drugs,[4] arrange necessary facilities for patients such as drinking water facility,[5] observe the empty blisters and update treatment card daily,[6] inform higher authority if a patient didn't come to DOTS even for one day.[1]

The main aim of any drug therapy is to achieve certain desired outcomes. However, despite of best drug therapy and effort of health professionals, those outcomes might not achieve if the patients are non-compliance.[7] Literature from various part of the world on definition of TB treatment noncompliance show wide variation.

2. Methodology

The study was conducted at DCC of Kalutara. DCC Kalutara provide services for the entire population (i.e., 1.3 million in a 1600km^2) of Kalutara district. Every TB patient reported in Kalutara District need to visit the DCC-Kalutara for registration, and for initial medical examination and follow-up. The present, descriptive cross-sectional study was conducted from January 2018 to October 2018. In this study, "noncompliance" was defined as missing or interrupting of taking anti TB drugs consecutively or intermittently for four days or more per week, within intensive phase. Any patient who interrupt talking anti TB drugs consecutively or intermittently for less than four days per week considered as compliance patient.

All confirmed TB patients registered at District Tuberculosis Register (DTR) of Kalutara District from 2017 October 1st to 2018 March 31st were selected ($n = 304$) as the study population. Under aged TB cases (age less than 18 years), retreatment TB cases and extended TB cases were excluded. Total of 267 TB patients were eligible for the study. Once patients complete the intensive phase, interviewers administered questionnaire (i.e., structured, pre-tested) was used to collect date from all consented TB patients at DCC. Interviewers visited their homes and administer the questionnaire for non-compliant patients not attending DCC. Questionnaires were included several socio-demographic factors such as age, gender, marital status, ethnicity, religion, education, status of living environment, status of employment, average monthly income, etc. Further, questionnaires ascertained information on behaviors such as current consumption of alcohol and smoking status. Characteristics and practices of DOTS centers (e.g., availability of drinking water facility, checking of empty blisters of TB drug card) and side effects were also inquired.

The relevant secondary data were abstracted from DTR, TB treatment card and TB follow up card at the DCC. This information was important to identify the noncompliance patients and the compliance patients. Once the data entered, categorical data were summarized as frequency and proportions with relevant measures of dispersions. Significance level was taken as 0.05 to assess the association. The study protocol was

approved by the Ethics Review Committee of the Postgraduate Institute of Medicine, University of Colombo.

3. Result

There were total of 267 eligible patients during the data collection period; 15 (5.6%) patients were not responded. Out of the responded patients (n = 252), 46 (18.3%) were identified as noncompliant TB patients. Educational level (p = 0.038), living environment (p = 0.004) and absent of care giver (p = 0.031) were significantly associated with noncompliance for TB treatment (Table 1).

Majority of TB patients (n = 218, 86.5%) visited DOTS provider weekly while rest of the patients (13.5%, n = 34) visited daily to take anti-TB drugs (Table 2). Majority of DOTS providers (61.9%, n = 156) did not observe for drug intake and did not check the empty blisters (73.4%, n = 185) (Table 2). Drinking water facility were not available in most of the DOTS centers (67.1%, n = 169) (Table 2). Noncompliance for TB treatment was associated with "lack of observation for drug intake by the DOTS provider" (p = 0.028) (Table 3).

Table 1 – Association between sociodemographic factors of participants and their noncompliance for TB treatment in intensive phase.

Sociodemographic factors of participants	Compliant n (%)	Non-compliant n (%)	Total n (%)	Significance χ^2, p
Age[a]				
18–59	134 (81.2%)	31 (18.8%)	165 (100)	
60 and above	72 ((82.8%)	15 (17.2%)	87 (100)	$\chi^2 = 0.091$, p = 0.762
Gender				
Male	131 (81.9%)	29 (18.1%)	160 (100)	
Female	75 (81.5%)	17 (18.5%)	92 (100)	$\chi^2 = 0.0049$, p = 0.944
Marital status				
Married and living together	160 (82.5%)	34 (17.5%)	160 (100)	
Single/Separated/Widowed	46 (79.3%)	12 (20.7%)	58 (100)	$\chi^2 = 0.2995$, p = 0.584
Ethnicity				
Sinhalese	124 (86.1%)	20 (13.9%)	144 (100)	
Tamil	40 (78.4%)	11 (21.6%)	51 (100)	
Muslim	40 (72.7%)	15 (27.3%)	55 (100)	
Burgher	02 (100%)	00 (00.0%)	02 (100)	$\chi^2 = 5.6589$, p = 0.129
Religion				
Buddhism	116 (86.6%)	18 (13.4%)	134 (100)	
Hindu	33 (78.6%)	09 (21.4%)	42 (100)	
Islam	40 (72.7%)	15 (27.3%)	55 (100)	
Christian	17 (80.9%)	04 (19.1%)	21 (100)	$\chi^2 = 5.3778$, p = 0.146
Highest Educational achievement				
Ordinary level or below	180 (80.0%)	45 (20.0%)	225 (100)	
Above Ordinary Level	26 (96.3%)	01 (03.7%)	27 (100)	**χ^2=4.2904, p=0.038**
Living Environment				
Urban sector-Residential area	118 (85.5%)	20 (14.5%)	138 (100)	
Urban sector-Slum area	07 (63.6%)	04 (36.4%)	11 (100)	
Rural sector	62 (86.1%)	10 (13.9%)	72 (100)	
Estate sector	19 (61.3%)	12 (38.7%)	31 (100)	**χ^2=13.3383, p=0.004**
Employment status				
Employed	97 (80.2%)	24 (19.8%)	121 (100)	
Unemployed	109 (83.2%)	22 (16.8%)	131 (100)	$\chi^2 = 0.3898$, p = 0.532
Average monthly income (Rupees)				
No income	82 (77.4%)	24 (22.6%)	106 (100)	
0-15 000	17 (80.9%)	04 (19.1%)	21 (100)	
15 000–30 000	63 (82.9%)	13 (17.1%)	76 (100)	
>30 000	44 (89.8%)	05 (10.2%)	49 (100)	$\chi^2 = 3.5715$, p = 0.312
Care giver				
Present	191 (83.4%)	38 (16.6%)	229 (100)	
Absent or living alone	15 (65.2%)	08 (34.8%)	23 (100)	**χ^2=4.6339, p=0.031**
Alcohol intake				
Yes	62 (79.5%)	16 (20.5%)	78 (100)	
No	144 (82.8%)	30 (17.2%)	174 (100)	$\chi^2 = 0.3863$, p = 0.534
Smoking				
Yes	32 (72.7%)	12 (27.3%)	44 (100)	
No	174 (83.6%)	34 (16.4%)	208 (100)	$\chi^2 = 2.9058$, p = 0.088
Total	**206(81.7%)**	**46(18.3%)**	**252(100)**	

[a] Study population divided in to two categories according to the age, 18y to 59 y group consider as adult and 60y and above group consider as senior adults.

Table 2 – Characteristics and practices of DOTS centers based on the respondents.

Characteristics and practices of DOTS centers	Number	Percentage (%)
How they provided DOTS		
Visit DOTS provider daily	34	13.5
Visit DOTS provider weekly	218	86.5
Availability of drinking water facility in DOTS center		
Available	83	32.9
Not available	169	67.1
Observation of drug intake		
Observed	96	38.1
Not observed	156	61.9
DOT provider checked empty blisters		
Yes	67	26.6
No	185	73.4
Total	252	100.0

Any adverse drug reactions, patients experienced after taking anti-TB drugs which were severe enough to get assistance from health care providers considered as positive for adverse reactions. Nausea or vomiting, dyspepsia or gastritis, headache, and liver injury were most common side effects patients experienced during the course of anti-TB treatment. Adverse drug reactions were associated with noncompliance for TB treatment ($p = 0.042$) (Table 3).

4. Discussion

TB require long term treatment while control mainly depend on completion of total treatment course successfully.[8] Noncompliance to the TB treatment is main barrier contributing for worsening the situation by increasing incidence and drug resistance.[9] The present study was based on TB patients registered in Kalutara DCC; the focal center for the third highest number of TB patients in Sri Lanka. Reporting of almost one fifth of non-compliant patients out of registered TB patients, assure that a study of this nature generates important information for future service delivery.

As shown by previous studies, there is high variability in noncompliance rate within the country. As an example, present study reported the noncompliance of 18.3% in Kalutara district while the same figure in Colombo district in Sri Lanka was 23%.[10]

Non-compliance of TB patients is associated with education level ($p = 0.038$) in present study. This is similar to studies in other countries.[11] Probably, the higher education level lead to understanding of disease, consequences and the importance of compliance.

Living in a crowded and poorly ventilated area (e.g., slums, temporary houses at tea and rubber estate, prisons and refugee camps) is a known risk factor for the TB. Perhaps, these environments are favorable for growth of TB bacteria.[1] According to the results, present study showed an association between living environment and noncompliance ($p = 0.004$). In fact, high TB incident as well as poor compliance lead to difficulty in controlling TB in those areas. Studies of similar nature have shown the same results.[12]

Presence of a care giver is important for compliance since he/she supervise the drug intake. Absence of a care giver associated with noncompliance of TB treatment in the present study ($p = 0.031$) and reported in similar findings in other studies as well.[13]

Noncompliance for TB treatment is associated with male gender[8,14], unemployment[14] low financial level,[15] alcohol intake[16] in other studies. Nevertheless, none of these variables were associated with noncompliance for TB treatment in our study.

DOTS is proven to be important in TB control as compared to the self-administered therapy.[6] However, a study done in India found no statistically significant difference between success rate in patients taking DOTS and self-administered therapy.[17] Even though the national policy of Sri Lanka recommended implementing daily DOTS to all new PTB and Extra PTB[1] in intensive phase, present study results show that only 13.5% TB

Table 3 – Association between characteristics, practices of DOTS center, adverse reactions and noncompliance to TB treatment in intensive phase.

	Compliant, n (%)	Non-compliant, n (%)	Total, n (%)	Significance, χ^2, p
How they provided DOTS				
Visit DOTS provider daily	30 (88.2%)	04 (11.8%)	34 (100)	$\chi^2 = 1.1091$, $p = 0.292$
Visit DOTS provider weekly	176 (80.7%)	42 (19.3%)	218 (100)	
Availability of drinking water facility in DOTS center				
Available	73 (67.8%)	10 (15.2%)	83 (100)	$\chi^2 = 3.1942$, $p = 0.073$
Not available	133 (138.2%)	36 (30.8%)	169 (100)	
Observation of drug intake				
DOTS provider observed the drug intake	85 (88.5%)	11 (11.5%)	96 (100)	**χ^2=4.799 p=0.028**
DOTS provider did not observe the drug intake	121 (77.6%)	35 (22.4%)	156 (100)	
DOT provider checked empty blisters				
Yes	57 (85.1%)	10 (14.9%)	67 (100)	$\chi^2 = 0.6776$, $p = 0.410$
No	149 (80.5%)	36 (19.5%)	185 (100)	
Adverse drug reaction				
Yes	58 (74.4%)	20 (25.6%)	78 (100)	**χ^2=4.131, p=0.042**
No	148 (85.1%)	26 (14.9%)	174 (100)	
Total	206 (81.7%)	46 (18.3%)	252 (100)	

patients visited DOTS center daily and rest of TB patients visited DOTS center weekly. Ideally, patients should go to DOTS center daily to take drugs and DOTS provider should observe the patients while swallowing drugs in daily basis.[1] To facilitate drug intake, drinking water facility should be available in DOTS centers. However, only one third of patients confirmed the availability of drinking water facility in their respective DOTS center. Similar findings have reported from India as well.[18]

In this study only 38.1% of patients reported that they have taken anti-TB drugs in a DOTS center while drug intake was observed by a DOTS provider. However, the national DOTS policy and the WHO guidelines recommend to swallow the drugs under the supervision of a DOTS provider, mandatorily.[1] However, this issue has reported in other countries with various extents. As an example, India reported direct observation of drug intake practice ranged from 26.4% to 82.4%.[18] Importantly, our study showed lack of observing the drug intake by DOTS provider was associated with noncompliance ($p = 0.03$).

Checking the empty blister of TB drugs is a part of DOT providers' duty to ensure the accuracy of information provided by patients.[1] However only 26.6% of patients confirmed that empty blisters have checked by DOTS provider (Table 2). Our study revealed, presence of side effects due to anti TB drugs was associated with noncompliance (Table 3). Some studies in other countries also revealed the same result[8],[19].

Our research has several limitations. Firstly, the treatment noncompliance information was based on self-reported information of patients, which may bias by patients' recall. Secondly the study was conducted on patients registered over a period of six months. Although the study would have ascertained the important information related to the noncompliance in Kalutara District, it would have more representative if a longer period is considered.

5. Conclusions

Noncompliance for TB treatment during the intensive phase is still a challenging problem. The noncompliance was associated with not observing the drug intake by DOTS providers, side effects of the drugs, educational level, living environment and absent of care giver. DOTS was not followed as recommended in guidelines. Therefore, suitable interventions should be arranged among DOTS providers.

Declarations ethical approval and consent to participate

The research proposal was approved by Ethics Review Committee of the Post Graduate Institute of Medicine, University of Colombo, Sri Lanka. Written informed consents were taken before interviews.

Consent to publish

Administrative authorities consented the collection and publication of data. All authors read the manuscript and agreed to publish.

Availability of data and materials

The datasets analyzed during the current study available from the corresponding author on reasonable request and with the permission of Ethics Review Committee, Post Graduate Institute of Medicine, University of Colombo, Sri Lanka.

Conflicts of interest

The authors have none to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijtb.2020.09.018.

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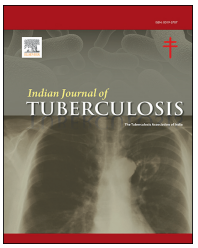

Review Article

Thyroid tuberculosis

Lalit Kumar Bansal [a], *Stuti Gupta* [b], *Arun Kumar Gupta* [c], *Poras Chaudhary* [a,*]

[a] *Department of General Surgery, Dr Ram Manohar Lohia Hospital and Post Graduate, Institute of Medical Education and Research, New Delhi, India*

[b] *Department of Lab Medicine, All India Institute of Medical Sciences, New Delhi, India*

[c] *Department of Surgery, Dr Ram Manohar Lohia Hospital and Post Graduate, Institute of Medical Education and Research, New Delhi, India*

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ABSTRACT

Thyroid tuberculosis is a rare disease. Its incidence is low even in countries where prevalence of pulmonary tuberculosis is high (0.1–0.4%). In literature, there are only a few cases which were diagnosed as thyroid tuberculosis. It can be explained by a high resistance of the thyroid gland to infectious processes. However, the prevalence of tuberculosis has increased worldwide and thyroid involvement can be a primary manifestation of the disease. The incidence of extrapulmonary tuberculosis has been showing a progressive increase in the recent years(Barnes and Weatherstone, 1979). The most frequent clinical presentation is a solitary thyroid nodule that may present as a cystic nodule. It may also present as thyroid abscess with pain, fever and other non-specific signs and symptoms. ATT results in complete cure therefore it is important to differentiate it from other form of thyroiditis. Patients are usually euthyroid, but cases of hypothyroidism and hyperthyroidism are described. For accurate diagnosis of thyroid tuberculosis, clinical and radiological features are nonspecific and histological examination is required for confirmation of diagnosis. PCR may help in diagnosis. The authors encounter 3 cases of thyroid tuberculosis in last 5 year which are described in this article. The aim of this study is to review all the cases published in literature to describe clinical presentation, appropriate diagnostic method and possible treatment options of the disease.

1. Introduction

Thyroid tuberculosis is extremely rare condition, but should be considered as differential diagnosis of thyroiditis with previous history of tuberculosis or history of contact with TB patient, especially in countries, where there is a high prevalence of tuberculosis.[1] There are only a few published case reports and case series on thyroid tuberculosis. Thyroid tuberculosis is an uncommon manifestation of one of the most common infections, caused by *Mycobacterium*

* Corresponding author
E-mail addresses: dr.lkbansal@gmail.com (L.K. Bansal), ranustuti@gmail.com (S. Gupta), drguptaarunkumar@gmail.com (A.K. Gupta), drporaschaudhary@yahoo.com (P. Chaudhary).

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tuberculosis. *Mycobacterium tuberculosis* is an aerobic bacillus responsible for most cases of tuberculous infection. Approximately one-third of the world's population are estimated to be infected.[2] TB usually affects the lungs (in 90%) but in 15–20% of cases it affects other sites, most commonly lymph nodes,[3] bone and joints, pleura, the central nervous system (especially in immunocompromised individuals), peritoneum, the gastrointestinal or genitourinary tracts and the pericardium. Organs such as the heart, thyroid and pancreas and tissues such as striated muscle are relatively resistant to TB infection.[4–6] The aim of this study is to review all the cases published in literature to describe clinical presentation, appropriate diagnostic method and possible treatment options of the disease. Authors encountered 3 cases of thyroid tuberculosis, out of them 2 cases were diagnosed preoperatively on FNAC examination and one case diagnosed on histopathological examination after thyroidectomy.

1.1. *Method*

Electronic search was undertaken in MEDLINE and PUBMED using the terms "thyroid" in combination with "tuberculosis". Total of 63 studies were identified in various case reports and series. All available articles are reviewed. History and case series are placed in tabulated form. Pathogenesis, clinical features, diagnosis and treatment were summarized and concluded.

1.1.1. *History*

Albers, in 1847, is accredited by Foerster as being the first to have observed tuberculosis in the thyroid gland.[1]

Lebert, in I857, reported his observations made at necropsy of involvement of the thyroid gland in a fatal case of miliary tuberculosis.[2]

Four years later Rokitanski made the statement that tuberculosis never involves the thyroid gland, and Virchow was of the opinion that by some antagonistic action the association of goitre and tuberculosis is prevented.[7]

Quinlan, in 1874, first suggested that the specific changes in the thyroid gland which could regularly be found in acute miliary tuberculosis could also be found in the chronic forms of pulmonary tuberculosis.[6]

In 1878 Chiari described 7 cases of microscopic involvement of thyroid in patients who died from disseminated tuberculosis.[1]

Bruns et al in 1893, reported first confirmed case in which the diagnosis was established at operation. A woman, forty-one year of age, with compressive symptoms of enlarging thyroid, underwent thyroidectomy. The gland removed at operation was studied by Baumgarten, who saw proliferating follicular epithelium take part in the formation of tubercles by transformation into giant cells.[8]

The first report of successful drainage of tuberculous thyroid abscess was by Schwartz in 1894.[9]

Morin, in 1895, described sclerosis of the thyroid gland which he frequently observed following death from pulmonary tuberculosis. He reported 348 such cases. Morin observed that patients even with slight enlargement of the thyroid gland showed a greater tendency to improvement and eventual healing.[1]

Kashiwamura,[10] in 1901, studied the thyroid gland at necropsy in a large number of cases of patients who had died of infectious diseases. Although he noted sclerosis almost invariably in all cases of chronic tuberculosis, He also demonstrated identical sclerosis of the interstitial tissue in typhus, diphtheria, and in a case of gas poisoning.

The publications of Poncet and Keriche, in 1906, and their school of thought popularized the conception of a close relation between tuberculosis and goitre.

Rankin and Graham in 1932 from the Mayo Clinic in 20,758 cases of tuberculosis reported an incidence rate of tuberculous thyroid in 0.1% of their patients.[1]

El Malki HO, et al, diagnosed 8 thyroid tuberculosis cases in 2426 partial thyroidectomy specimens, an incidence of 0.3%.[11]

In a study conducted by Das et al the incidence of tuberculous thyroiditis was 0.6% among 1283 thyroid lesions subjected to aspiration cytology.[12]

Rokitansky found only 21 cases of thyroid tuberculosis out of 20,758 surgically resected thyroid glands (incidence 0.1%).[13]

In 20th and 21st century, many cases were reported, some of which are discussed in table 1.

2. Epidemiological features

Mycobacterium tuberculosis is an aerobic bacillus responsible for most cases of tuberculous infection. Approximately one-third of the world's population is estimated to be infected.[2] In 2012, 1.4 billion deaths were due to mycobacterium tuberculosis which is found to be the second largest cause of death due to an infectious disease after HIV.[2] The highest incidence is in Asia and Africa with 40% of the world's cases occurring in India and China.[3] Between 5% and 10% of people infected with mycobacterial tuberculosis develop symptoms within their lifetime (higher rates are seen in individuals co infected with HIV).[2,3] TB usually affects the lungs (in 90%) but in 15–20% of cases it affects sites outside the lungs (extrapulmonary TB), most commonly lymph nodes.[3]

Primary TB of the thyroid gland is an extremely rare extrapulmonary manifestation of TB even in areas where TB prevalence is very high.[7] It has been estimated to occur at a frequency of 0.1–0.4% of all TB cases.[1] This is highlighted in a study by Mondal et al, who described only 18 cases of thyroid TB out of 1565 patients who underwent fine-needle aspiration cytology (FNAC) of thyroid lesions over 9 years, 4 of whom had known pulmonary TB. India is a country with the high burden of tuberculosis pulmonary and extrapulmonary. The World Health Organization in 2015 gives an estimated incidence of 2.2 million cases of TB in India, the global incidence is 9.6 million. In 2015, the estimated prevalence of TB in India was 2.5 million.[14]

According to a study conducted by Rankin et al, in 1932, more than 95 per cent of the cases, it was possible to identify the factors such as age, sex, presence or absence of tuberculosis elsewhere in the body, type of operation employed and the pathological description of the thyroid tissue removed.[1] There were 83 women and 17 men. In four instances the sex was not given. The average age of the women was thirty-six and a half years, and of the men, it was twenty-six years.

The incidence of tuberculosis among organ transplant recipient is estimated to be 20–74 times more than the general

Table 1 – Various cases of thyroid tuberculosis described in literature.

Author/year	Total No. of cases	Clinical presentation	Imaging	investigation	FNAC	Histopathology
Rankin et al 1932	104	Mostly with miliary tuberculosis, 6 thyroid tuberculous abscess, 94 subtotal thyroidectomy cases	None	21 cases suspicious for hyperthyroidism	Not done as all cases diagnosed post op on histopathology	Tubercle and giant cell in most cases
Das et al 1992	8	Age ranged from 14 to 65 years. Six patients presented with clinically palpable nodule; two patients presented with neck abscess	USG: 4 solitary nodules, 2 extra thyroidal lesions, 1 cystic isthmic lesion, and 1 case not imaged	None reported	Of 1283 thyroid aspirates over 2 years, 8 (0.6%) diagnosed tuberculosis Five AFB-positive aspirates	None reported
Khan et al 1993	4	Case 1: thyrotoxicosis Case 2: thyroid sinus tract Case 3: dysphagia, fever Case 4: progressive thyroid enlargement	Case 2: USG show right hypoechoic nodule Case 3: USG shows multiple hypoechoic nodules	Case 1: elevated T4 Case 3: elevated ESR (115 mm/h)	Cases 2–4: Epitheloid granulomas	Coalescing, caseating epitheloid granulomas, giant cells detected in surgical specimen
Mondal and Patra 1995	18	Age ranged from 36 to 52 years. Three cases with cervical lymphadenopathy, Four cases with pulmonary tuberculosis	Iodine thyroid scan: all cases demonstrated solitary nodules	Thyroid function reported normal in all cases Elevated ESR in 4 cases	Of 1565 thyroid aspirates over 9 years, 18 cases (1.15%) noted tuberculous thyroiditis	All cases demonstrated epitheloid granulomas with necrosis
Pazaitou et al 2002	3	One case presented with generalized symptoms (weight loss, diaphoresis) 2 has nonspecific symptoms	Iodine thyroid scan: 2 cases demonstrating cold thyroid nodules	Thyroid function reported normal in all cases ESR >100 mm/h in all cases	One aspirate yielded white fluid, positive AFB stain One aspirate yielded lymphocytes	Each thyroidectomy specimen was AFB positive
Bulbuloglu et al 2006	49	10 cases- abscess, 2 cases- cyst, 10 cases- extrapulmonary TB	USG- hypoechoic thyroid in most cases	Thyroid function normal in all cases	Not done	Epitheloid granuloma
Ghosh et al 2007	200	Neck mass, abscess or cyst formation	USG: heterogeneous hypoechoic mass	Thyroid function reported normal In most cases	Pus or serous material	Epitheloid granuloma/Langhan's cell/caseous necrosis

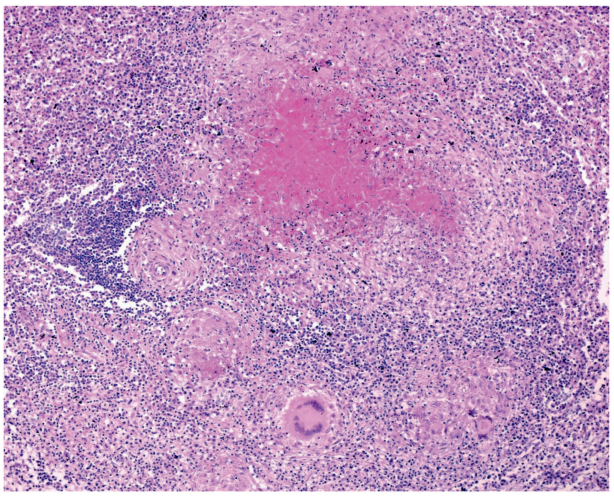

Fig. 1 – Histopathological picture showing caseating granuloma.

population.[15] The incidence among renal transplant recipients was 0.35%–1.2% in the United States, 0.7%–5% in Europe, 3.1%–5% in Southeast Asia, and 5%–15% in India and Pakistan.[15] The incidence of TB in south India in pretransplant patients is 8.7%, and that in renal allograft recipients is 12.3%.[16] The reported incidence from North Indian center is similar.

Most frequently, the patients are middle-aged women. In the literature, the mean age at onset was around the third to the fourth decades with a slight female predominance.[5]

2.1. *Etiology*

Thyroid gland is relatively resistant to tubercular infection. Some risk factors such as age, diabetes mellitus, malnutrition, and acquired immunodeficiency syndrome were associated with the occurrence of tuberculosis of the thyroid gland.[17]

The specific microscopical changes peculiar to tuberculosis have been produced experimentally in the thyroid glands of animals. Different experimental studies done by Roger and Germier, Torri, Tomellini, Shimodiara and others on rabbit, guinea pigs and mice.[1] They concluded that the colloid material in the glands possesses a bactericidal action and also bacilli became attenuated, after prolonged contact with colloid. Plummer et al are of the opinion that the relationship between hyperthyroidism and tuberculosis of the thyroid gland is not a coincidence. They stated: "Either a hypertrophic gland is rendered more susceptible to invasion by the bacillus of tuberculosis or the infection stimulates the parenchyma to abnormal activity" and is thus indirectly responsible for the hyperthyroidism with its attendant symptoms". Plummer et al, in 1920, further stressed this association and reported hyperthyroidism in five of their seven cases.[1]

2.1.1. *Pathogenesis*

Some investigators considered that the spread ways for thyroid TB might include miliary spread, direct spread from an adjacent focus, such as cervical lymph nodes tuberculosis, laryngeal tuberculosis, tracheal tuberculosis, mediastinal lymph node tuberculosis, and lymphatic spread.[18] Thyroid tuberculosis may have different clinical manifestations and may be difficult to diagnose. In the thyroid gland, the tuberculous involvement may be in two main forms. First, which is more common, is miliary spread to the thyroid gland as a part of generalized dissemination. Less common is focal caseous tuberculosis of thyroid, presenting as a localized swelling mimicking carcinoma,[4] as cold abscess appearing superficially,[19] as multinodular goiter,[20] or very rarely as an acute abscess. Thyroid tuberculosis can also manifest itself as a common thyroid nodule or lump or as a nodule with a cystic component.[21] It was once considered immune from the disease till Lebert in 1862 reported the involvement of the gland in a patient with disseminated tuberculosis.[22] The probable reasons for the relative immunity of thyroid gland from tuberculosis are the bactericidal attribute of the colloid, extensive vascularity and high iodine content of the gland.[23]

2.1.1.1. *Pathology*. The pathology of thyroid tuberculosis may be in following forms.[24–26]:

a) Multiple lesions throughout the gland like miliary tuberculosis
b) Enlargement of gland due to caseating granulomas (Fig. 1)
c) Cold abscess formation sometimes with multiple sinuses
d) Chronic fibrosing tuberculosis, difficult to distinguish from De Quervein's thyroiditis
e) Acute abscess formation, when there is a danger of making wrong diagnosis of carcinoma.

The most important and characteristic features of tuberculous inflammation are necrotizing epithelioid cell granulomas with Langhan's type giant cells. Demonstration of acid-fast bacilli by ZN staining confirms the diagnosis, but this stain is often negative in tissue sections.[24,25]

Microscopically four morphological variations of tuberculous thyroiditis have been distinguished.[27]:

1. Multiple tubercles in case of miliary TB
2. Solitary and sometimes merging tubercles
3. Foci of caseous necrosis or cold abscess, and
4. Cicatrized tubercle foci.

3. Clinical presentation

The clinical features are often mild, but it may be severe in case of abscess or thyroiditis.[6] Patients may be asymptomatic. The constitutional symptoms of tuberculosis such as fever, weight loss, night sweats, and anorexia may be present.

Bulbuloglu et al reviewed 76 thyroid TB cases reported from 1905 to 2004 and found that 49 cases were presented most commonly with solitary nodules or multinodular goiter, 10 cases with abscess, 2 cases with cyst, and only 10 cases with coexistent pulmonary tuberculosis.[28]

Ghosh et al reviewed about 200 Thyroid TB cases reported before 2006 and found that almost all cases had primary foci elsewhere in the body, and isolated Thyroid TB was extremely rare.[29] The patient may have symptoms of dysphonia,

dysphagia, breathing difficulties, and rarely recurrent laryngeal nerve paralysis due to expanding gland.[30] Patients are usually euthyroid and cases with thyroid hormones abnormalities are extremely rare. Presence of thyrotoxicosis due to tuberculous thyroiditis is reported as "a clinical syndrome of hyperthyroidism" which occurs in the initial period of glandular involvement due to its destruction.[31] The hypothyroidism may be present due to extensive glandular destruction by caseous necrosis. In the literature, only three cases of hypothyroidism due to thyroid TB have been reported yet.[32] In our case series 2 case have features of pain and fever with local signs of abscess formation. One case has severe pain, with fever with pressure symptom for which hemithyroidectomy was done.

3.1. *Diagnosis*

Diagnosis of thyroid tuberculosis is difficult because of its rare occurrence. A past history or contact with tuberculosis patient with cervical lymphadenopathy and thyroid enlargement may lead to correct clinical suspicion and diagnosis. If mycobacterial infection is suspected, a chest X-ray and a tuberculin skin test (PPD) can be performed.[33] When attempting to prove or deny presence of tuberculous infection in the thyroid, it is necessary to apply most of methods used to diagnose tuberculosis, from the most simple (such as chest X-ray and PPD test)[34] to the most modern rapid test like serological assays, when antibodies to *Mycobacterium tuberculosis* are detected in human serum or plasma (ELISA).

In order to identify tuberculosis infection, it should be found in sufficient concentration in the examined material, and this concentration is different for each diagnostic method. Cytological investigation allows to detect *Mycobacterium tuberculosis* provided there are not less than 10,000 microorganisms in each ml of the examined material, culture from the investigated material is effective when there are not less than 50 tuberculous bacilli in each ml. Biological test (infecting susceptible animals with tuberculosis) are informative in case when there are 1–5 such bacilli in each ml. Polymerases chain reaction gives a positive result when each ml of the tested material contains solitary bodies of microorganisms.[35]

The diagnosis is possible only after fine-needle aspiration cytology (FNAC) or after histopathological examination of the surgical specimen if FNAC is negative or inconclusive.[36] The histological findings are epithelioid cell granulomas with central caseous necrosis. Other histological findings which are characteristic are peripheral lymphocytic infiltration, and Langhan's giant cells.[24] Caseous necrosis is very specific to tuberculosis. Microscopic demonstration of acid-fast bacilli (AFB) confirms the diagnosis. Nowadays, it is stated that acid fast bacilli are not always found, therefore, multiple coalesced and caseated epithelioid cell granulomas along with giant cells are considered to be diagnostic of tuberculous affection of the gland. However, it is obligatory to stress that this is the specific reply of human organism to the tuberculous agent. Massive use of antibacterial drugs has caused alteration of the course of tuberculosis and adjustment of mycobacterium to antituberculous drugs, therefore, the immune response of human organism to this agent may be non-specific. In the case

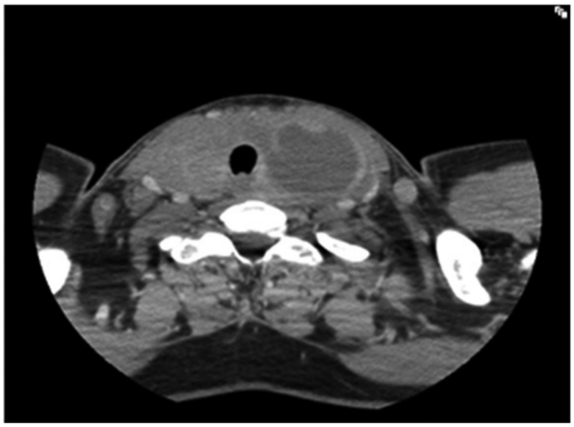

Fig. 2 – CECT picture showing thyroid abscess with necrosis.

only common features characteristic to inflammation can be found in any organ, including the thyroid gland.[10]

Mycobacterial culture may also be helpful but it takes time to give result and also sensitivity is very low.

The imaging techniques are not very helpful because of non-specific finding of disease and also of rare occurrence.[25] Ultrasonography findings are heterogenous, hypoechoic lesion in thyroid gland. Abscess is anechoic and may show internal echoes.[25] CECT may help to localize the abscess with central necrotic picture[35] (Fig. 2). Peripheral-enhancing low-density abscess with regional lymphadenopathy can be demonstrated on CT scan. In MRI study normal thyroid gland is homogenously hyperintense relative to the neck muscles on both T1 and T2 weighted images. In thyroid tuberculosis intermediate signal intensity due to the presence of densely cellular inflammatory granulation tissue, with tuberculous granulomas with or without minimal necrosis can be seen.[37] But these findings are nonspecific and can be seen in other condition of thyroiditis and abscess formation.

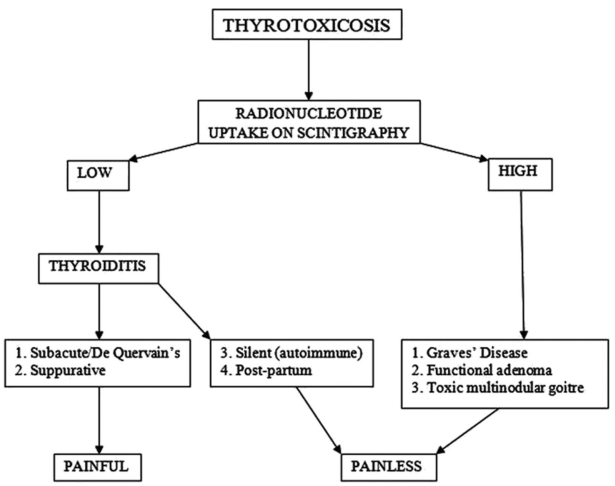

Fig. 3 – Flow diagram showing different uptake in thyroiditis and other condition of hyperthyroidism in radionuclide scan.

Thyroid scintigraphy is useful to differentiate between thyroiditis and other forms of hyperthyroidism based on the uptake of radionucleotide (Fig. 3). The preferred radiopharmaceutical agent is iodine-123 but technetium-99m sodium pertechnetate is a suitable alternative with lower radiation exposure.

In a patient with thyroid swelling, nonspecific clinical presentation and inconclusive imaging studies, following criteria can be used to diagnose thyroid tuberculosis.

1. Previous history of pulmonary or extrapulmonary tuberculosis or contact history with tuberculosis patient
2. Serological essay demonstrating antibody against mycobacterium tuberculosis
3. Thyroiditis with abscess formation
4. Caseous necrosis with or without granuloma formation and Langhan's type cell in FNAC specimen of thyroid gland

If any 3 from above mentioned criteria are found in any case, diagnosis of thyroid tuberculosis can be safely made and treatment can be started accordingly.

In our case series 2 cases have caseous necrosis with Langhan's type giant cell with abscess formation with history of contact with tuberculosis patient.

4. Differential diagnosis

Thyroid tuberculosis should be differentiated from other infectious diseases of the thyroid and abscess. Local pain is the predominant clinical finding in infectious form of thyroiditis and sub-acute granulomatous thyroiditis (De Quervain's, thyroid sarcoidosis, etc.).[37] Other differential diagnosis that can be present with granuloma are granulomatous thyroiditis, palpation thyroiditis, fungal thyroiditis, sarcoidosis, granulomatous vasculitis, and foreign body reaction. Caseation necrosis is very specific and can be seen only in tuberculous inflammation. Thyroid tuberculosis might be falsely diagnosed as thyroid malignancy if pain is absent or both conditions may even coexist.[38] A differentiation from thyroid cancer is essential to avoid unnecessary thyroid surgery Table 1.

4.1. *Treatment*

Thyroid tuberculosis is treated by antituberculous drugs like any other extrapulmonary tuberculosis. If it is complicated by abscess formation, surgical drainage is required.[39] Standard four drug regime treatment for 6 months is sufficient in most cases but in cases with large abscess, surgical drainage or resection followed by antituberculous treatment is considered sufficient, and further surgery is rarely required.[40] The British Thoracic Association demonstrated that a regimen of H or INH and R for 6 months, supplemented during the first 2 months with Z or PZA and E, was as effective as the 9-month regimen of INH and R with E in the first 2 months. So, the UK and WHO recommendation for thyroid tuberculosis is 2 months of INH, R, Z and E followed by 4 months of INH and R. The US recommendation is 2 months of INH, R, Z and E followed by 7 months of INH and R. A Drug Susceptibility Testing should always be performed.[41] Treatment failure is rare and is usually due to bacterial resistance to standard antituberculous therapy. Other available regimen can be useful in resistance cases. In summary antitubercular drugs are sufficient to treat thyroid tuberculosis. Surgical indications are –

1. Abscess formation single or both lobe of thyroid
2. Treatment failure due to resistance or noncompliance
3. Pressure symptoms like dysphagia, dysphonia, respiratory problem, laryngeal nerve paralysis
4. Suspicion of malignancy

In our study, out of 3 cases, 2 cases were diagnosed on FNAC and resolved completely by antitubercular therapy alone. In one case abscess was formed in the right lobe of thyroid which has nonspecific finding on FNAC and imaging studies. Due to unsatisfactory response to conservative therapy, right lobe thyroidectomy was done. On histopathological examination it turned out to be tubercular etiology for which patient discharged on antitubercular therapy.

5. Conclusion and recommendations

Thyroid tuberculosis has been an important problem since 19th century. Tuberculous infection spreads to the thyroid by lymphatic, hematogenous route or directly from adjacent organs. Thyroid tuberculosis is an extremely rare condition, but should be considered as differential diagnosis of thyroiditis with previous history of tuberculosis or history of contact with TB patient especially in countries, where there is a high prevalence of tuberculosis. The data revealed marked predominance of female patients evenly distributed over the 3rd and 4th decades. The mechanisms underlying the relative resistance of the thyroid gland to TB infection are unknown. Factors such as the thyroid capsule, high iodine levels within the gland, bactericidal action of the colloid, antitubercular action of thyroid hormones and the abundant lymphatic and vascular supply to the thyroid gland may explain why primary TB at this site is seldom encountered. The prevailing opinion is that probably all cases are secondary to some disease process elsewhere in the body. The clinical features are often mild, but it may be severe in case of abscess or thyroiditis. Patients may be asymptomatic or non-specific symptoms like fever, weight loss, night sweats, and anorexia may be present. Past history of tuberculosis, presence of cervical lymphadenopathy and high ESR values may help in the diagnosis, but thyroid tuberculosis can occur even in the absence of these features. FNAC is the main diagnostic method to diagnose the disease. Acid-fast bacilli staining and culture from aspirated thyroid material sometimes are insufficient to detect tuberculosis. Therefore, the newest and the most modern tests may be necessary to identify them. Imaging study can help in diagnosis but findings are very nonspecific. The principal syndrome exhibited by these patients is hyperthyroidism. Moreover, the question of whether the hypertrophic gland is rendered more susceptible to invasion by the bacillus of tuberculosis or the infection stimulates the parenchyma to abnormal activity, and is thus indirectly responsible for the hyperthyroidism, could not be conclusively determined. Thyroid tuberculosis should be differentiated from all the main diseases of the thyroid. It is

particularly important to distinguish it from thyroid cancer in order to avoid unnecessary thyroid surgery. The treatment is mainly based on the antituberculous agents, but surgery or drainage may be required for large abscess, treatment failure, pressure symptoms or suspicion of malignancy followed by antituberculous drug therapy.

Declaration of Competing Interest

All authors have none to declare.

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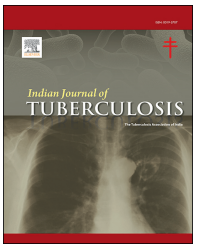

Correspondence

Roaming homeless persons, India—Pulmonary tuberculosis☆

Approximately there are 1.8 million homeless people in India; 52% of them live in urban cities (IGH 2018).[1] There are very few reports on the prevalence of pulmonary tuberculosis among homeless in India.[2,3]

Recently we are conducting a study of screening homeless persons (with no fixed abode-Roaming homeless) for pulmonary tuberculosis in Chennai city, India. This screening was undertaken in all zone of Chennai city. Permission for conducting the study is given from the concern Municipal Corporation and police department authorities of Chennai City. The screening was done at place and time to the availability of homeless persons. One medical officer and other supporting staff with mobile digital x-ray unit formed the survey team. Digital mobile x ray unit was used to take Digital chest —X- ray, Posterior-Anterior view, onsite. Two samples (one spot, one on next day early morning or two on spot) of sputum specimens was collected from persons with the sign and symptoms; cough for more than two weeks, recent weight loss, fever, haemoptysis, chest pain, history of pulmonary tuberculosis and radiological abnormalities suggestive of pulmonary tuberculosis. Sputum smears were examined under florescent microscopy for Acid Fast Bacilli and culture on Lowenstein—Jensen medium for the detection of *mycobacterium tuberculosis*.

The number of bacterial positive pulmonary tuberculosis cases detected were 16 in 1024 screened roaming homeless persons and estimated to 1524 per 1,00,000 population. This is which 4.4 times more than general population of the same area, Chennai city[4] and 9 times more than the national target (170 per 1,00,000) to achieve by 2020 of Indian population.[5] Spread of the disease by roaming persons is likely, as they do not have stable habitat and were non-compliant with treatment follow up.[6]

In order to achieve set targets for reduction in tuberculosis (TB) morbidity and mortality (TB free India by 2025),[5] the National TB program must work on public health emergency for roaming homeless persons to achieve the targets through reduction in tuberculosis incidence in India. Using of mobile teams for screening can be a feasible option with finger print based identification of registration of roaming homeless persons like Unique Identification numbers, "Aadhaar"[7] and offering incentives for treatment.[8]

Conflicts of interest

The authors have none to declare.

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Chandra Kumar Dolla*
Epidemiology Dept, ICMR-NIRT, India

C. Padma Priyadarshini
P.K. Bhavani
Clinical Dept, ICMR-NIRT, India

T. Kannan
Statistics Dept, ICMR-NIRT, India

P. Kumaravel
Epidemiology Dept, ICMR-NIRT, India

K. Devika
Bacteriology Dept, ICMR-NIRT, India

**Corresponding author*. Tel.: +91 44 28369663, +91 9884783385(mobile).
E-mail address: ckdolla@gmail.com

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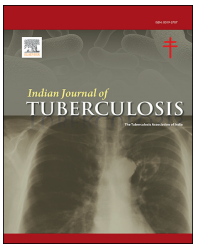

Correspondence

Kluver-Bucy syndrome: A rare aftermath of tubercular meningitis

Keywords:
Hyperorality
Hypersexuality
Visual agnosia

Dear Editor,

We present a nine-year-old boy, who completed the course of anti-tubercular therapy (ATT) and developed core symptoms of Kluver-Bucy syndrome (KBS).

9-year-old male child presented with hyperorality, hyperactivity, impulsiveness, inattentiveness, and hypersexuality for past one year. Parents also complain that the boy had difficulty in recognizing familiar objects and faces. These symptoms were gradual in onset and progressive causing significant mental distress to the parents. There was no history of trauma, rash, joint pains, altered sensorium, fever, seizures, cognitive decline and focal neurological deficits. The child was a known case of tubercular meningitis (TBM), completed a full course of anti-tubercular therapy one year back. Considering clinical presentation of the child, possibility of Kluver-bucy syndrome, Non-convulsive status epilepticus, temporal lobe epilepsy, Isoniazid-ethambutol induced psychosis, autoimmune encephalitis, Kleine Levin syndrome were considered. Magnetic Resonance imaging (MRI) brain and electroencephalography (awake and sleep) were normal. Drug induced psychosis was ruled out as the ATT was stopped one year back. Absence of hypersomnia and cognitive changes make Kleine-Levin syndrome less likely. Hence a probable diagnosis of KBS was made and the patient was started on carbamazepine along with behavioural therapies with significant improvement in the symptoms.

Tubercular meningitis (TBM) is the most common form of central nervous system (CNS) tuberculosis and has very high morbidity and mortality.[1] In addition to the common complications such as hydrocephalus, other rare manifestations like generalized myoclonus, rigidity, ataxia and KBS have also been reported in children. KBS is a relatively rare behavioral phenomenon that appears most often after bilateral temporal lobe damage. Main features of KBS include hyperorality, hypersexuality (frequent holding of genitals, rubbing of genitals to bed after lying prone), placidity, visual agnosia, and hypermetamorphosis (excessive exploration of the environment).[1,2] The pathophysiology of KBS is thought to occur due to the disturbances in the temporal portions of limbic networks that connect with multiple cortical and subcortical networks primarily involving amygdale, uncus, hippocampus, insular cortex, orbitofrontal and cingulate gyri.[2,3] Various etiologies implicated include herpes simplex encephalitis, traumatic brain injury, TBM, juvenile neuronal ceroid lipofuscinosis, temporal lobe epilepsy, systemic lupus erythematosus, neurocysticercosis, anti NMDAR encephalitis and listeria meningoencephalitis.[3,5] Most of the pediatric patients present only few features of the disease described as 'partial kluver Bucy syndrome'. The diagnosis is mainly clinical and also requires meticulous exclusion of all the differentials. MRI brain is essential to know the extent of damage of temporal lobe, but in few cases MRI can be normal, most probably functional MRI could give a better picture of temporal lobe affection. . Management includes carbamazepine and leuprolide to reduce abnormal sexual behaviours, haloperidol, risperidone and fluoxetine to treat other hyperactive behaviours.[2–5] Often, the management is not satisfactory and residual behaviour abnormalities tends to persist.

Statistical analysis

Not applicable.

Consent

Written informed consent obtained from parents.

Ethical statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Authors' contribution

SSJ and BS: Patient management, literature review, and initial draft manuscript preparation;
PM, LS: Patient management, literature review, and critical review of manuscript for important intellectual content.
AS: provided inputs regarding psychiatric issues.
All authors were involved in patient management and approved the final version for publication.

Conflicts of interest

The authors have none to declare.

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Suchit S. Jogu
Department of Pediatrics, Advanced Pediatrics Centre, Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh, 160012, India

Bhanudeep Singanamalla
Priyanka Madaan
Pediatric Neurology Unit, Department of Pediatrics, Advanced Pediatrics Centre, Post Graduate Institute of Medical Education and Research, Chandigarh, 160012, India

Akhilesh Sharma
Department of Psychiatry, PGIMER, Chandigarh, 160012, India

Lokesh Saini*
Pediatric Neurology Unit, Department of Pediatrics, Advanced Pediatrics Centre, Post Graduate Institute of Medical Education and Research, Chandigarh, 160012, India

**Corresponding author*. Tel.: +9968859725.
E-mail address: drlokeshsaini@gmail.com

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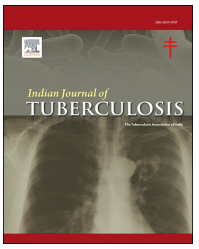

Correspondence

Uprooted by COVID pandemic: National TB elimination programme needs acceleration!

Keywords:
Tuberculosis
COVID
India

In India, the global pandemic of COVID first made its presence during March 2020.[1] The country has taken several measures to contain the spread of pandemic which includes lock-down of the entire country for nearly forty days. As such, all the vibrant lifesaving activities of National Health programme had come to a record low levels. The country being one of the highest tuberculosis burden globally had committed to eliminate TB by 2025. A remarkable dent has been caused by COVID pandemic on the ongoing TB control activities across the country. The routine programmatic activities like case-finding, initiation of treatment, follow-up and contact tracing is worst affected. Anecdotal evidences suggest that the country has registered only 40–50% of TB cases when compared to the same period during last year. The well-known reasons include involvement of the entire health systems in the region for containment and management of COVID patients and at the same time the community is skeptical about using the health facilities with the fear of contracting the corona virus infection when they approach health facility while the rapid TB diagnostic services were completely stopped during the period. In this context, we discuss the strategies that programme should adopt that will help programme to gain momentum to reach the elimination target.

First, boosting the morality of programme staff and health care workers to execute their functions effectively despite the difficult field conditions. The health system should ensure continuous supply of appropriate personal protective equipment at the institution.[2] The programme should develop a policy document, guidelines and robust supervision and monitoring tools for collection of sputum at microscopy centres with strict emphasis on implementation of infection control practices. These centres has potential of becoming a source of COVID infection for presumptive case of TB attending the health facility; hence, utmost care needs to be taken. Second, incentivized online training of all the health care providers at public and private health facilities. The health care workers have to be educated regarding the facts and myths of corona virus infection and the possible ways of handling a case of presumptive TB and patients of TB.[3] A mobile application from Government of India called "Aarogya Setu" has to be installed which alerts the person if they come across a person with corona virus infection.[4] Third, minimizing the visit of person with presumptive TB and patients of TB at health facilities and promoting "Home based care for TB diagnostic and treatment services". The programme should develop newer effective and innovative mechanisms for teleconsultations, sputum collection and delivery of anti-TB drugs with an inbuilt enhanced supervision and monitoring tools. This strategy can be effectively implemented in urban areas and it is expected to bring down the patients visit to health facilities by 80%. However, the challenge for the programme is to make bold investments in technologies which are swift and sustainable. Fourth, the programme should consider operational research to study the impact of corona virus infection on TB and its associated co-morbidities and their treatment outcomes. To conclude, tuberculosis is a preventable and curable disease any laxity in implementing the said strategies will significantly bring down the programme's momentum that were gathered in the last few years.

Conflicts of interest

The authors have none to declare.

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Sharath Burugina Nagaraja*
ESIC Medical College and PGIMSR, Bengaluru, Karnataka, India

Santosha Kelamane
Independent Public Health Consultant, Hyderabad, Telangana, India

Shilpashree Madhava Kunjathur
BGS Global Institute of Medical Sciences, Bengaluru, Karnataka, India

Sanath Kumar Gurram Krishnamurthy
District Tuberculosis Officer, Tumkur, Karnataka, India

**Corresponding author*. Tel.: +91 9480147083.
E-mail address: sharathbn@yahoo.com

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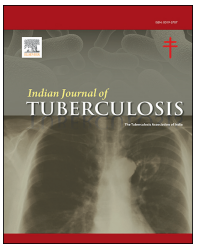

Viewpoint

Care of tuberculosis patients in the times of COVID-19

Sudheer Tale[*], *Pahel Meitei Soibam*

Department of Pulmonary Medicine, AIIMS, Rishikesh, India

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ABSTRACT

Globally during this time of Covid-19 pandemic health care services are overhelmed and it has negative impact on other diseases like Tuberculosis (TB). High TB burden countries like India despite being faced by several other problems in present times, is continuously trying to provide uninterrupted services to TB patients through the national programs. In this general perspective we have shared our opinion on problems faced by TB patients in the times of covid-19

First reported in China, the Covid 19 Pandemic now affects the whole world. As on May 31, 2020 there are more than 5,934,936 laboratory confirmed cases globally and with 3,67,166 deaths reported thus far.[1] This COVID 19 pandemic has affected virtually all aspects of care of non-Covid 19 patients, including patients with tuberculosis. Out of fear and because of forced restrictions on movement for non-emergency cases, many patients with tuberculosis do not step out of homes. To top it, a large number of people in the lower economic strata have lost their means of livelihood.

According to global TB report 2019 by World Health Organization (WHO), there were 10 million new tuberculosis cases globally in the year 2018, out of which 27% was contributed by India. Globally, there were 5 lakhs cases of DR-TB and out of which 27% was contributed by India. A total of 4.1 lakhs people died in India of tuberculosis in 2018 as per WHO.[2] To decrease the magnitude of disease burden in the country and hence a serious intent of the Government of India to eradicate the disease were both borne out by the change in the name of RNTCP (Revised National Tuberculosis Control Program) to NTEP (National Tuberculosis Elimination Program) on 1st of January 2020.

The Covid 19 pandemic, however, poses many challenges to the nation's endeavor for elimination of tuberculosis. First, follow up and care of the patients already on Anti-Tubercular Treatment (ATT) are compromised with. Because of the restricted movement imposed, timely management of drug related adverse events is being hampered and it may lead to adverse health consequences including death or patients may stop the treatment on their own. Secondly, mortality among patients with tuberculosis is also likely to be higher since COVID 19 disease is likely to take a more severe course in this group of patients, who, on top of their compromised lungs, happen to be chronically debilitated and malnourished. Third, the chaotic interstate reverse migration of laborers to their native places with the perils of transit and quarantine on one hand, and resultant lack of timely access to follow up or emergency facilities are a problem compounded by the

* *Corresponding author*. Tel.: +8872156777.
E-mail address: drtalesudheer@gmail.com (S. Tale).
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curtailment of health care services not related to Covid 19. It is likely to result in a huge surge in the number of treatment defaulters. It will result in the short term increase the death rate among patients with tuberculosis. On a longer term, it will lead to emergence of Multi drug resistant (MDR) tuberculosis. Fourth, detection rate of new TB cases will be reduced as patients don't report to health care facility due to fear of Covid-19 and as laboratory services for detection tuberculosis have been curtailed. This means delayed treatment, and more open cases of pulmonary tuberculosis who can infect other people in their family and community. Fifth, the number of untreated active cases will be multiplied because of the double whammy of increased open cases and decreased detection rate of new cases with additional increase in TB deaths in near future.[3]

While it is the collective responsibility of the Government, the health administrators and healthcare providers to ensure that all efforts are in place for continued pursuance of the goals of NTEP, we need to particularly emphasize how individual hospitals and the NTEP network can adapt to the goals of NTEP in these challenging times.

Hospitals can make exceptional provisions for continued testing of samples for tuberculosis detection, and for continued care either through telemedicine or through emergency medical services. District Tuberculosis Officers (DTOs) under NTEP can ensure that extra supply of ATT is given to migrant patients to cover the transit and quarantine periods as well. Registrations can be transferred to the nearest Directly observed treatment, short-course (DOTS) center of the respective native places of the patients to ensure continuity of treatment. The entire NTEP network can be adequately sensitized to ensure that release and receipt of registration of patients at the DOTS Centers under them happen without undue hassles to the patients and those patients currently on treatment are actively traced and encouraged to continue taking the treatment. The DTOs can ensure that patients registered with the various DOTS centers are contacted through calls or phone messages with clear instructions to get an extra supply of antitubercular drugs if they are going back to their native places, not to stop taking treatment and to take further supply of ATT from the DOTS centers to which their registrations have been transferred. Provisions can be made to allow patient's relatives to collect antitubercular medications from the DOTS centers with proper identity verification. Offices of the DTOs can consider opening telemedicine helplines for catering to the needs of patients with tuberculosis. For already diagnosed drug sensitive tuberculosis patients, interim policy can be formulated whereby the TB Health Visitor (TBHV) or DOTS provider will give full course of 4 drugs (H/R/Z/E) for intensive phase (IP) at a time to the patient and they can be advised to report to the nearest DOTS center after 2 months for assessment of clinical condition and for switch-over to the continuation phase (4 months of H/R/E). Meanwhile, if there is any clinical deterioration of health condition or drug related adverse effects, patients can be guided accordingly through telemedicine run by the DTOs' Offices or the nearest Hospital.

As we are moving towards an injection free regimen for treatment of MDR-TB patients, these patients can also be provided with MDR-TB treatment refills to align with scheduled clinical visits at the health facility. Patients already on or starting an all oral DR-TB regimen and on linezolid should be enquired about side effects like neuropathy and intensive monitoring of the complete blood counts (CBC) should be done in the first 2 months of treatment. Patients can consult their respective DOTS center or a nearby health care facility by telemedicine if they have any untoward side effects. An MDR-TB patient can be monitored monthly during the intensive phase (IP) through telemedicine by TBHV from the nearest DOTS center or DTO Office. He/she can come to nearest DOTS Center at the end of IP for clinical assessment, sputum examination and switch-over to continuation phase (CP). Smear, culture and other genotypic tests like CBNAAT and Line Probe Assay (LPA) can be done from the nearest Government facility or from there can be transported to DTU if the patient smear is positive at the end of IP. As noted above, telephonic counseling remains critical in these groups of patients for successful completion of treatment. In the current situation, the importance of all oral drug regimens is all the more evident since access to healthcare personnel in the vicinity of the patient is going to be more limited than before.

If any DS or MDR-TB patient acquires COVID 19 infection, treatment of TB should be continued along with other supportive management. Importantly, the physician treating COVID 19 and MDR-TB co-infections should be aware of the potential side effects of the drugs in MDR regimen like QT prolongation since drugs like hydroxychloroquine used in the treatment of COVID 19 can cause further QT prolongation and lead to fatal cardiac arrhythmias.[4] Finally, all TB patients should be educated about COVID 19 precaution measures like hand washing, wearing a mask, maintaining social distancing and finally good nutrition and adherence to their treatment regimen. These short-term measures may be more labor and resource intensive. However, the long term gains in our fight to eliminate tuberculosis will be immense. Or else, we may end up losing the previous gains NTEP has so far achieved.

Conflicts of interest

All authors have none to declare.

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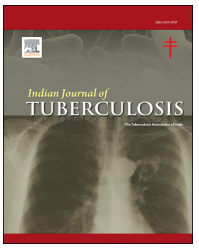

Viewpoint

Pretomanid: The latest USFDA-approved anti-tuberculosis drug

Uttiya Deb[*], *Supreeti Biswas*

Department of Pharmacology, Burdwan Medical College, Burdwan

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ABSTRACT

Pretomanid is a nitroimidazooxazine drug which inhibits synthesis of mycolic acid. This leads to defective cell wall formation, ultimately causing bacterial cell death. It is active against both replicating and non-replicating *M. tuberculosis*. Following promising result in a phase III trial, pretomanid was approved by United States Food and Drug Administration in August 2019. This orally active drug has been approved as part of a combination regimen of bedaquiline, pretomanid and linezolid (BPaL regimen) to treat adults with pulmonary extensive drug resistant tuberculosis (TB) or treatment-intolerant or non-responsive multi-drug resistant TB. Peripheral neuropathy and increased liver enzymes are some of the reported adverse events associated with pretomanid. However, more studies are required to confirm the role of pretomanid in paediatric, geriatric and HIV co-infection cases.

1. Introduction

Tuberculosis (TB) is a global disease caused by *Mycobacterium tuberculosis* infection. According to the Global TB report of World Health Organization (WHO) released in October 2019, an estimated 10 million people fell ill with TB in 2018 and a net estimated 1.2 million TB deaths occurred worldwide.[1] India accounted for 27% of global TB new cases identified in 2018.[1] TB produces drug-resistant strains which pose a serious therapeutic challenge to clinicians worldwide. Multidrug Resistance (MDR) is resistance to both isoniazid and rifampicin.[2] Extensive Drug Resistance (XDR) is resistance to isoniazid, rifampicin, fluoroquinolone and one of the three injectable second-line anti-TB drugs (amikacin, kanamycin or capreomycin).[2] There were 0.5 million new cases of rifampicin-resistant TB in 2018(of which 78% had MDR-TB).[1] The rapid emergence of resistant TB strains has led to decrease in the success rate of TB treatment. Added to it are the adverse effects of multiple anti-TB drugs administered at the same time and the result is a therapeutic nightmare. Successful research in anti-TB therapy has introduced newer anti-TB drugs bedaquiline[3] and delaminid[4] in recent past. With the approval of pretomanid by United States Food and Drug Administration (USFDA) in August 2019, we have a new anti-TB drug to tackle the global menace of TB.[5]

1.1. *Pretomanid*

Pretomanid is an orally active anti-mycobacterial agent which is to be administered as part of bedaquiline, pretomanid and linezolid (BPaL) regimen.[6] BPaL regimen, according to USFDA, has an efficacy rate of 89% which is much higher than 2017

* *Corresponding author*. Department of Pharmacology, Burdwan Medical College, Burdwan, 713104, India. Tel.: +91 342 2658646.
E-mail address: dr.uttiyadeb@gmail.com (U. Deb).

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global success rates (85% in TB, 56% in MDR-TB, 30% in XDR-TB).[1,5] Pretomanid has been approved for treatment of adults with pulmonary XDR-TB or treatment-intolerant or non-responsive MDR-TB. It has been approved by USFDA under the Limited Population Pathway for Antibacterial and Antifungal Drugs (LPAD).[7] It has also received USFDA's Qualified Infectious Disease Product (QIDP) designation which is given to specific antibacterial or antifungal products intended to treat serious or life-threatening infections in a limited population of patients with unmet needs.[8] QIDP status grant falls under the ambit of FDA Safety and Innovation Act (FDASIA).[9] Pretomanid is the second drug in USFDA history to receive QIDP status. It was also granted priority review status[10] and orphan drug[11] designation before its final approval. It is currently being developed by TB Alliance. Table 1 summarizes the important milestones in pretomanid development.

1.2. *Pharmacokinetics*

Food increases pretomanid absorption.[12] Mean maximum plasma concentration (C_{max}) and mean area under the plasma concentration curve (AUC $_{\infty}$) of pretomanid in fed state increases by 77% and 88% respectively in comparison to fasted state.[12] Peak plasma concentrations are obtained in a median 5.0 hours after administration. Pretomanid exhibit high plasma protein binding of approximately 86.4%. It has a mean volume of distribution of 97 litres after 200 mg single dose oral administration. Steady state concentration is achieved in 4–6 days after repeated oral administration of 200 mg pretomanid once daily.[12] The mean plasma half-life of pretomanid is 17.4 hours.

Pretomanid is metabolized by multiple reductive and oxidative pathways. Approximately 20% metabolism occurs via Cytochrome P450 3A enzyme. Metabolites of pretomanid are excreted predominantly via urine (53%) and faeces (38%), with <1% excreted in unchanged form. Data regarding dose modification based on patient age, renal or hepatic impairment is not yet available. There is no clinically significant impact of bodyweight, race, gender, HIV status and pulmonary TB status (XDR, treatment intolerant or non-responsive MDR) on pharmacokinetics of pretomanid.[12]

1.3. *Pharmacodynamics*

Pretomanid is a nitroimidazooxazine drug which inhibits synthesis of mycolic acid. It kills actively replicating *M. tuberculosis* by blocking bacterial cell wall production and thereby facilitates increased drug penetration into the bacterial cell.[13] This ultimately leads to bacterial cell death. It also kills non-replicating bacteria under anaerobic conditions by releasing nitric oxide.[13,14] Pretomanid is a prodrug which is metabolized to highly reactive nitro compounds like nitric oxide by the bacterial reductase enzyme deazaflavin-dependent nitroreductase (Ddn) and reduced form of cofactor F_{420}.[15,16]

1.4. *In vitro and in vivo activity*

Pretomanid has demonstrated high anti-TB activity compared to other anti-TB drugs, with a minimum inhibitory concentration (MIC) ranging from 0.06 to 1 mcg/ml.[17] MIC_{90} of pretomanid against MDR TB and XDR TB isolates was 0.063 mcg/ml.[18] Research on murine models of TB showed lesser relapse rate at 2 months and 3 months post therapy by 3-drug combination regimen of pretomanid, bedaquiline and linezolid compared to 2-drug regimen of any two of these three drugs.[19]

1.5. *Dosage and administration*

Pretomanid is currently recommended to be administered in combination with bedaquiline and linezolid (BPaL regimen) in pulmonary XDR-TB or treatment-intolerant or non-responsive MDR-TB.[6] Table 2 summarizes the dosage and duration of BPaL regimen. It is recommended to be administered as directly observed therapy and the combination regimen is to be taken with food.[12] Dose adjustments of linezolid to 600 mg daily or

Table 1 – Key milestones in development of pretomanid (as part of combination regimen).

Year	Development	
2005	Phase I trial initiated	
2007	Orphan drug status granted in USA and European Union	
2012	Phase II trial commences	
2015	Phase III trial commences(Nix-TB trial – NCT02333799)	
2019	March	NDA accepted for review
	April	MAA accepted for review in European Union
		Priority review status granted in USA
	August	Approved in USA

NDA, New Drug Application; *MAA*, Marketing Authorisation Application.

Table 2 – Current recommended dosing schedule of BPaL regimen.

Drug	Dosage form	Route	Dosage strength & duration
Pretomanid	Tablet	Oral	200 mg once daily X 26 weeks
Bedaquiline	Tablet	Oral	400 mg once daily X 2 weeks followed by 200 mg once daily thrice/week X 24 weeks
Linezolid	Tablet	Oral	1200 mg once daily X 26 weeks

300 mg daily is to be considered in cases presenting with known linezolid adverse reactions of myelosuppression, peripheral neuropathy and optic neuropathy. BPaL regimen may be extended beyond 26 weeks if required.

1.6. Adverse events and necessary precautions

The most common adverse events observed in clinical trials on pretomanid were peripheral neuropathy, acne, anaemia, nausea, vomiting, musculoskeletal pain, and increased liver enzymes (transaminases and gamma-glutamyltransferase).[12,20] Pretomanid used in combination with bedaquiline and linezolid should not be used in patients with hypersensitivity to bedaquiline or linezolid. QT prolongation is a known adverse drug reaction of bedaquiline.[21,22] Precautionary measures like monitoring of serum potassium, calcium and magnesium should be taken while administering BPaL regimen.[12,23] Also, the regimen should be stopped if QT interval is more than 500 milliseconds or patient develops clinically significant ventricular arrhythmia.[12]

Liver function tests should be done at baseline, at two weeks and then monthly onwards while on treatment with BPaL regimen. Care should be taken to avoid alcohol and concomitant administration of other hepatotoxic agents. Complete blood counts should be constantly monitored to eliminate occurrence of myelosuppression (including anaemia, leukopenia, thrombocytopenia, and pancytopenia).[12,24] Visual function should be monitored to identify incidence of optic neuropathy. Monitoring of bicarbonate and lactic acid levels should also be considered in patients with suspected lactic acidosis as a result of linezolid.[24]

1.7. Drug interactions

Pretomanid should not be administered with other CYP 3A4 inducers like rifampicin, efavirenz.[12] It can be co-administered with protease inhibitors lopinavir/ritonavir.[12,25] Pretomanid significantly inhibits organic anion transporter 3 (OAT3) drug transporters in vitro; however, clinical drug–drug interactions with OAT3 substrates have not been conducted.[12]

1.8. Pretomanid resistance

Pretomanid resistance have been associated with mutations in five *M. tuberculosis* genes (ddn, fgd1, fbiA, fbiB, and fbiC).[26,27] These genes are involved in activation of pretomanid within the bacterial cell. Not all isolates with increased minimum inhibitory concentrations (MICs) have mutations in these genes, suggesting the existence of at least one other mechanism of resistance. The frequency of pretomanid resistance ranges from $1/10^7$ to $1/10^5$ organisms at 2 to 6 times the pretomanid MIC.[12]

1.9. Pretomanid in special populations

There is no data regarding pretomanid use in pregnancy.[12] Animal studies have shown post-implantation loss in the presence of maternal toxicity (including reduced bodyweight and feed consumption). Rat studies have shown that pretomanid is concentrated in breast milk but it is not known whether pretomanid and its metabolites are excreted in human milk. Safety and effectiveness of pretomanid have not yet been established in paediatric patients. Clinical studies on

Table 3 – Ongoing clinical trials on pretomanid (PA-824).

Title	Intervention	Phase	ClinicalTrials.gov identifier
Single-Dose Study to Evaluate the PKs of Pretomanid in Subjects With Renal Impairment Compared to Subjects With Normal Renal Function	PA-824	I	NCT03896750
Safety and Efficacy of Various Doses and Treatment Durations of Linezolid Plus Bedaquiline and Pretomanid in Participants With Pulmonary TB, XDR-TB, Pre- XDR-TB or Non-responsive/Intolerant MDR-TB (ZeNix)	Pretomanid, Linezolid, Bedaquiline, Placebo	III	NCT03086486
Pragmatic Clinical Trial for a More Effective Concise and Less Toxic MDR-TB Treatment Regimen(s) (TB-PRACTECAL)	Bedaquiline, Pretomanid, Moxifloxacin, Linezolid, Clofazimine	II/III	NCT02589782
Trial to Evaluate the Efficacy, Safety and Tolerability of BPaMZ in Drug-Sensitive (DS-TB) Adult Patients and Drug-Resistant (DR-TB) Adult Patients	Pretomanid, Bedaquiline, Moxifloxacin, Pyrazinamide, HRZE[a], HR[#]	II/III	NCT03338621
Assessing PA-824 for Tuberculosis (the APT Trial)	PA-824, Rifampin, Rifabutin, Pyrazinamide, Ethambutol, Isoniazid	II	NCT02256696
The Individualized M(X) Drug-resistant TB Treatment Strategy Study (InDEX)	Individualized TB treatment with multiple drugs, Standardized TB treatment with multiple drugs	IV	NCT03237182
Pharmacokinetic Study of Antiretroviral Drugs and Related Drugs During and After Pregnancy	Antiretroviral drugs(ARV) + Anti-TB, ARV, Anti-TB	IV	NCT00042289

[a] *HRZE*, Isoniazid, Rifampicin, Pyrazinamide, Ethambutol; *#HR*, Isoniazid, Rifampicin.

BPaL regimen did not include sufficient number of older patients (≥65 years) to determine geriatric dose modifications.

1.10. *Limitations of pretomanid use*

Pretomanid should not be used in the following cases:

1) Patients with drug-sensitive TB
2) Latent infection due to *M. tuberculosis*
3) Extra-pulmonary TB
4) MDR-TB that is not treatment-intolerant or nonresponsive to standard therapy

1.11. *Current status of pretomanid*

Pretomanid received its first approval on August 14, 2019 in USA as part of a combination regimen with bedaquiline and linezolid (BPaL regimen) for treatment of adults with pulmonary XDR or treatment-intolerant or non-responsive MDR TB.[28] It is currently under regulatory review in the European Union.[29]

USFDA approved pretomanid based on interim data from a single phase III trial (NCT02333799) conducted in three centres of South Africa.[30] In the study 88% of the patients suffering from XDR-TB or treatment-intolerant or non-responsive MDR-TB had no TB bacteria in sputum after completing the treatment(BPaL regimen) for 6 months.[31] Table 3 summarizes the ongoing clinical trials on pretomanid.

2. Conclusion

The World Health Organization's End TB strategy defines milestones for 2020 as a 35% reduction in TB deaths and a 20% reduction in TB incidence rate. The long term goals for 2030 are a 90% reduction in deaths due to TB and an 80% reduction in TB incidence rate.[1] The burgeoning menace of MDR-TB and XDR-TB are serious impediments in attaining these milestones. With the approval of pretomanid, the fight against TB gets a new arsenal which will help in eradicating TB. However, more studies are required to confirm the role of pretomanid in paediatric, geriatric and HIV co-infection cases.

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