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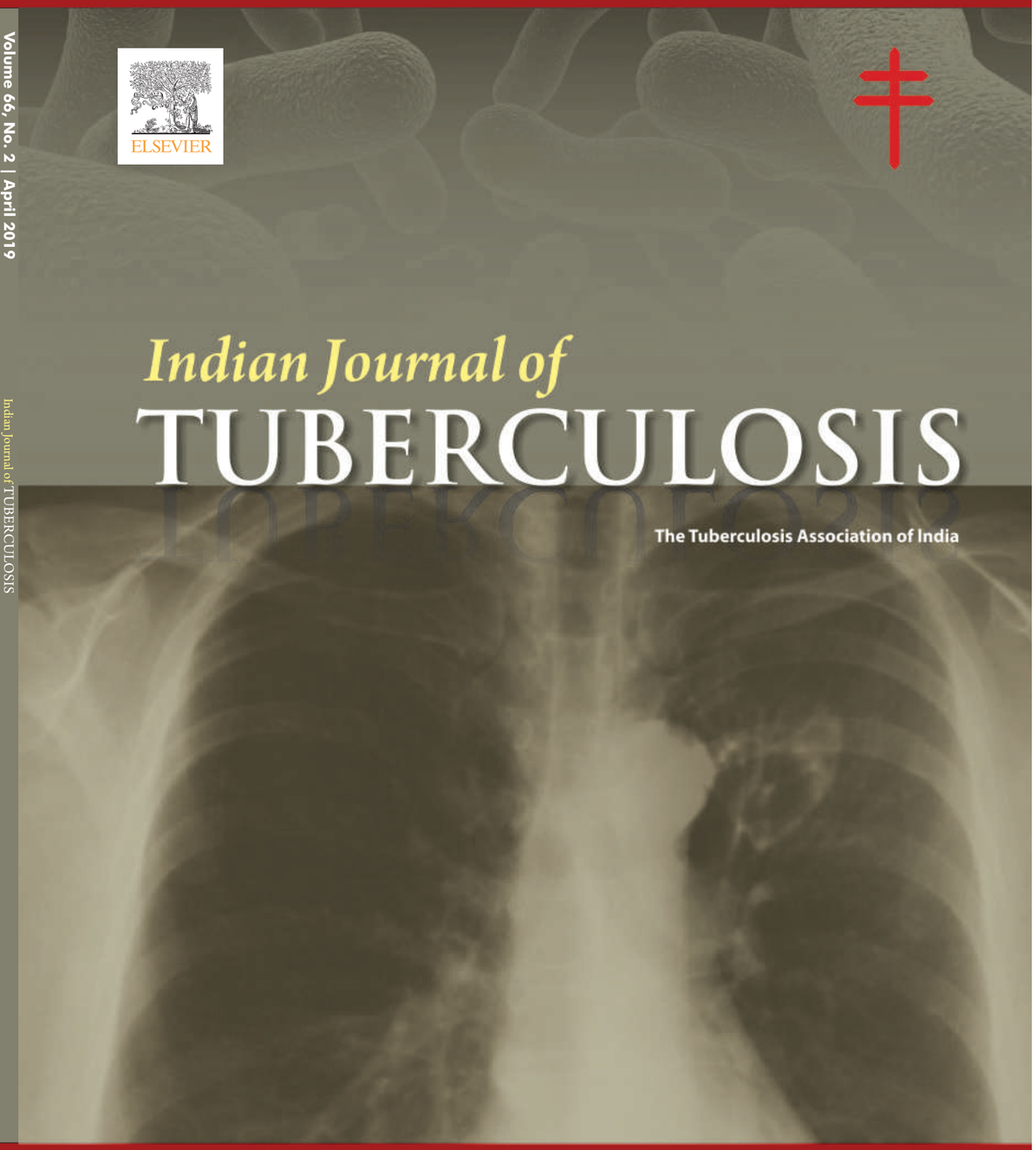

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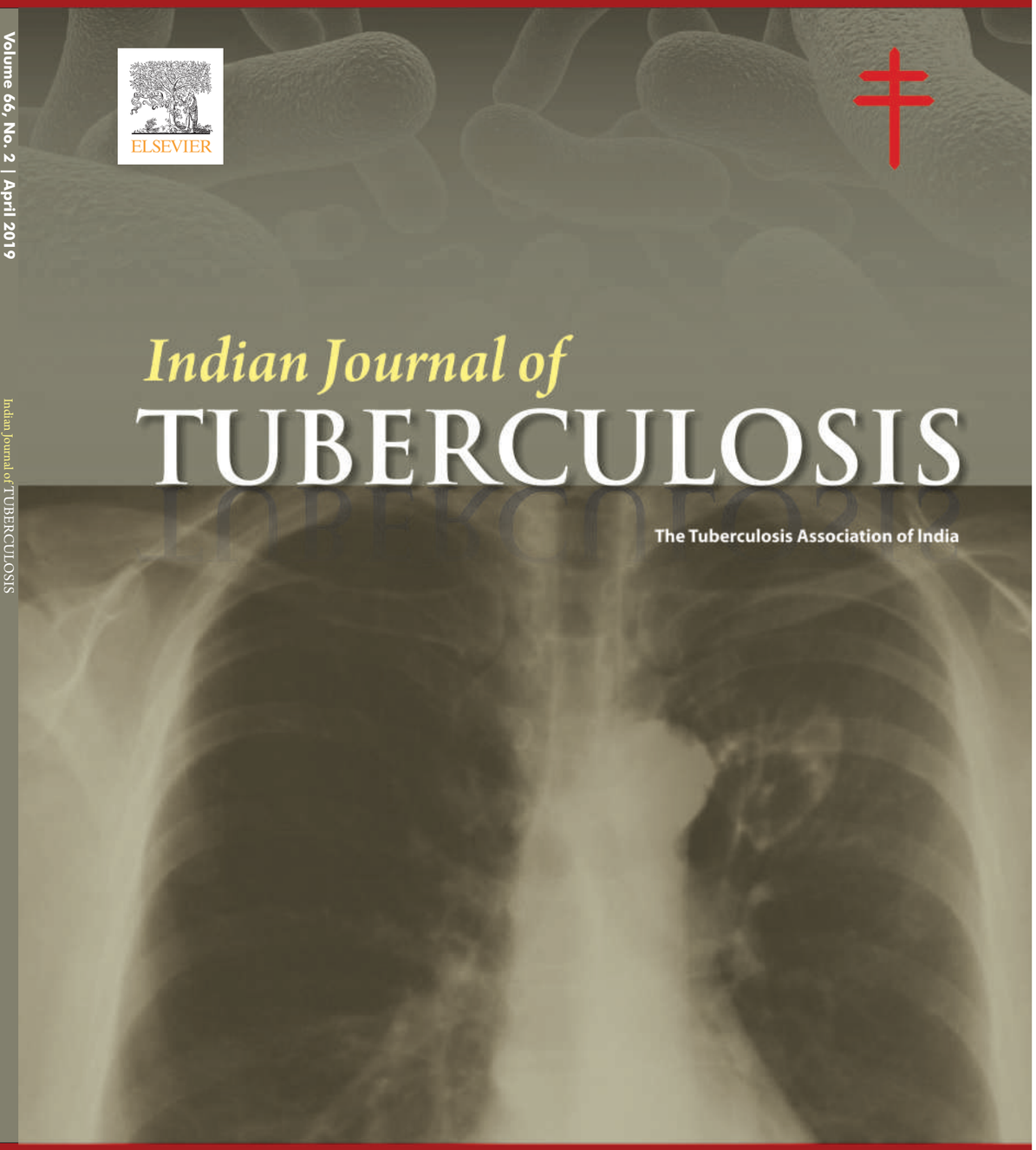

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Editorial

No families affected by tuberculosis to face catastrophic costs by 2020: India's new strategic plan to eliminate tuberculosis

The India's national strategic plan (NSP) for elimination of TB 2017 to 2025 is a framework to guide the activities of all stakeholders including the national and state governments. Its vision is to achieve 'TB Free India' with zero deaths, disease and poverty due to tuberculosis (TB). The main goal is to achieve a rapid decline in burden of TB, morbidity and mortality while working towards elimination of TB in India by 2025. In particular, a key outcome indicator is to achieve zero catastrophic cost for affected families.

Catastrophic health expenditure (CHE) is defined as out-of-pocket expenditure for health care that exceeds a specified proportion of household income (10–40%), with the consequence that the household may have to sacrifice the consumption of other goods and services necessary for their well-being.[1,2] Catastrophic health expenditure refers to any expenditure for medical treatment that can pose a threat towards a household's financial ability to maintain its subsistence needs. Total health expenditure of 10% or more from the total income is often considered an indication of CHE.[3] Dissaving (taking out loans or selling assets to finance health related expenditure)[4], is a convenient potential proxy for calculating catastrophic costs.

In this editorial, we discuss the factors that influence CHE and share our views on how to achieve zero catastrophic cost for TB affected families. We describe the interventions and strategies used and suggested by health programmes in reducing the catastrophic cost.

A growing number of studies from developing countries suggest that the health care payment has increased the poverty level and affects the poor most [9–11].[5,6,7] Globally, studies on economic impact due to TB on patient and their families on account of out of pocket expenditure and loss due to work absenteeism placed a high economic burden on households.[8,9] Compared to developed countries which are covered by public health systems or social health insurance, developing countries are over dependent on out of pocket spending on health.[10] Poor health is a common consequence of poverty and vice versa. Poor health leads to poverty through the inability to work and generate income. The more the disease progresses and complications occur, the more spending on medical treatment happens. For people with low income this is one of the devastating consequences of falling ill, and the burden can be enormous for those who do not have health insurance. Delay in diagnosis is quite common for TB disease due to lack of finance, accessibility, lack of severity of symptoms, many times people will not seek treatment until the disease has affected their daily activities. Further, financial shocks from medical expenses for treatment, households are often faced with income loss if affected members are working adults. Households with hospitalised members, with elderly, or chronically ill members, and those who use in patient services especially of private hospitals are more likely to face CHE. Factors that increase the likelihood of CHE are income, age of head of the household, children in the household, gender of the household head, and the level of education. A systematic review on financial burden for tuberculosis patients in low and middle income countries reported that cost as percentage of income was particularly high among poor people and those with multidrug resistant TB.

Over recent years, the World Health Organization (WHO) has promoted the concept of universal health coverage (UHC), emphasizing the need for access to services at an affordable cost to protect households from CHE. Reports on CHE due to TB in India are few[8,9,11] and it was reported that the proportion of various costs in relation to annual family income was 13% for direct costs, 26% for indirect costs, and 40% for total cost,[8] among patients whose income was below the poverty line, this proportion was 19% and among patients whose income was above the poverty line it was 10% respectively.[9] Although the economic burden of tuberculosis on households is known to be high, there is a paucity of information on financial risk protection strategies.

In a modelling study done for India and South Africa published in Lancet Global Health 2017, it was shown that catastrophic costs could be potentially averted (6–19%) by improvements in treatment for drug sensitive and multidrug

resistant TB.[12] In this model, for India, the authors assumed improvement in quality of treatment to mean increased treatment adherence from 75% to 85% for drug sensitive tuberculosis and from 48% to 67% for multidrug resistant tuberculosis. The intervention scenario described in their model included (1) improved quality of private sector treatment by provider training, supervision, regulation, and subsidies (2) provision of patient retention incentives (3) nutritional support and (4) linkage to social welfare programmes. For South Africa, the authors considered the following intervention scenarios (1) provision of mobile health care and follow-up in the community (2) offering patients adherence counselling and psychosocial support, and (3) tracing of patients to avert treatment dropout. Additional features to achieve improved treatment of multidrug-resistant tuberculosis would be (1) increased staffing and (2) decentralisation of the electronic register. In another modelling exercise published in the year 2016, on cost-effectiveness and resource implications of aggressive action on TB in China, India, and South Africa, a combined analysis of nine models has shown that the expansion of TB control programme would substantially reduce costs incurred by patients.[13] The assumptions used were (1) paying subsidies for TB care in the private sector (2) increasing microscopy access in the public sector (3) mobile screening units with symptom screen, X-ray, or Xpert algorithms (4) replacing smear by Xpert in routine diagnostic algorithm in the public sector (5) improving private sector quality through provider training, supervision, regulation, and subsidies (6) providing patient retention incentives (7) nutritional support and (8) linking to social welfare programmes. The experience from other national programmes is encouraging. It was shown that the National Health Mission (NHM) has shown a reduction in catastrophic health spending on maternal care from 56% in pre NHM period to 29% in post NHM period. NHM has been successful in increasing maternal care and reducing the catastrophic health spending.[14] Yet another suggestion by Madhukar Pai et al in their editorial on India's plan to eliminate TB by 2025 was that India must address the major gaps that have already been identified in the TB cascade of care in the public system and to address these gaps, they suggest that Revised National TB Control Programme (RNTCP) will need to modernise its TB standard of care and control by introducing and scaling up of rapid molecular diagnostics.[15]

A study from Chhattisgarh, India describes innovative social protection mechanisms for alleviating catastrophic expenses on multidrug resistant tuberculosis (MDR-TB) patients. A number of social protection schemes are being implemented by the central and state governments of India for improving TB patients' access to and affordability of health care. There are two health insurance coverage schemes Rashtriya Swasthya Bima Yojna (RSBY), Mukhyamantri Swasthya Bima Yojna (MSBY) having a Respiratory Medical TB insurance package to cover the expenses of seriously ill TB patients in Chhattisgarh. This innovative RSBY and MSBY MDR-TB insurance package is a step towards reducing catastrophic expenses associated with treatment for MDRTB.[16]

In addition to the above strategies in reducing CHE one can also consider (1) identifying the distribution of population below poverty within the communities and make focussed input in future programme implementation (2) addressing the barriers to access the unreached (3) focussing on the poor for early diagnosis and treatment (4) addressing the related social determinants in a holistic manner such as housing, livelihoods, risk behaviour, nutrition, food security and (5) extending the health sector to activities beyond their regular boundaries of action. This demands a stronger inter sectoral emphasis in the planning of TB programmes.[17].

The milestone that no families affected by TB face catastrophic costs implies minimizing direct medical costs, such as fees for consultations, hospitalization, tests and medicines as well as direct non-medical costs such as those for transport and any loss of income while under care. It requires that tuberculosis patients and TB affected households have access to appropriate social protection schemes that cover or compensate for direct non-medical costs and income losses. Introducing new digital technological interventions and novel approaches such as 99DOTS and video supported home based TB care, video observed therapy (VOT), eHealth portal could also reduce catastrophic costs to TB patients. With sufficient political commitment, TB related costs could be rapidly reduced in India and we may be able to reach the target by 2020.

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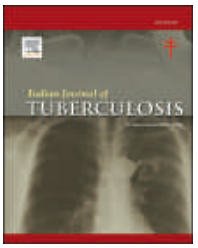

Original article

Pattern and trends of drug sensitivity in MDR-TB cases in Delhi (2009–2014): A record based study☆

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ABSTRACT

Background: This study was conducted with the objective to understand pattern and trends in drug sensitivity among MDR-TB cases in Delhi from 2009 to 2014 using existing records.
Methods: A retrospective record-based study was conducted at three Drug-resistant TB (DR-TB) treatment centers in Delhi. For data collection, patient treatment cards and TB registers were accessed. All multidrug-resistant (DR) TB patients registered, and initiated on treatment from January 2009 to December 2014 in three DR-TB centers were included in the study.
Results: A cumulative total of 2958 MDR-TB cases were registered in the three DR-TB centers during the period from Jan 2009 to December 2014. The median value time interval between culture test result and initiation of treatment was 82 days. High resistance was found against Streptomycin (70%), Ethambutol (40.1%), Ofloxacin (42.4%) and Kanamycin (12%). Favorable treatment outcomes ranged from 52.5% to 56.9% in cases resistant to only first-line anti-TB drugs but was much lower in the pre XDR-TB cases (26.3%) and XDR-TB cases (12.5%).
Conclusion: The proportion of cases with additional resistance to ofloxacin, kanamycin, ethionamide, and streptomycin increased over time from 2009 to 2014. Reducing the time to treatment initiation from initial diagnosis of MDR-TB could further improve treatment outcomes.

☆ All the authors contributed towards the intellectual content, drafting/editing of the article and approved the final manuscript being submitted.

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1. Introduction

Tuberculosis (TB) is a major global health problem and is a leading cause of death among infectious diseases, second only to HIV-AIDS. Global prospects for TB control are threatened by the emergence of strains which are drug-resistant, especially those that are multidrug-resistant (MDR) and extensively drug-resistant (XDR).[1] MDR-TB is defined as resistance to isoniazid (INH) and rifampicin (RMP) two of the most effective anti-TB drugs, with or without resistance to other drugs.[2] Extensively drug-resistant tuberculosis (XDR-TB) is defined as resistance to at least isoniazid and rifampicin, and to any fluoroquinolone, and to any of the three second-line injectables (amikacin, capreomycin, and kanamycin).[3]

The spread of multidrug-resistant tuberculosis (MDR-TB) and subsequent emergence of extensively drug-resistant tuberculosis is a daunting challenge for global TB control strategies.[4] The treatment for MDR-TB compared to drug sensitive TB requires prolonged treatment with multiple injectable drugs, more adverse effects, increased frequency of hospital admissions and much greater likelihood of treatment failure and mortality.[5]

In India, drug-resistant tuberculosis (DR-TB) cases are programmatically identified under the Revised National Tuberculosis Control Programme (RNTCP) and treated with the expanded DOTS-Plus regimen.[6] There is paucity of large sample studies from India which have examined drug sensitivity patterns in MDR-TB patients. Moreover, identifying the emerging pattern and trends of DR-TB is a critical pivot in the planning and implementation of effective TB prevention and treatment strategies. This study was thereby conducted with the objective of understanding the pattern and trends in drug sensitivity among MDR-TB cases treated in Delhi from 2009 to 2014 using the existing records from DR-TB treatment centers.

2. Materials and methods

A retrospective record-based study was conducted at three selected Drug-resistance TB (DR-TB) treatment centers in Delhi which were chosen purposively. The RNTCP introduced programmatic management of drug-resistant tuberculosis (PMDT) based on evidence-based guidelines in 2007 which was extended across India by 2013.[6] Since 2012, the conventional culture and drug susceptible testing (CDST) under the RNTCP is being complemented with the widespread scaling up of rapid molecular diagnostic tests like the Cartridge Based Nucleic Acid Amplification tests (CBNAAT) for detection of M.TB and Rifampicin resistance and the Line Probe Assay (LPA) test. These services were initially offered to only treatment failure cases. Subsequently, they are being provided to all presumptive MDR-TB cases which include treatment failure cases, contacts of index MDR-TB cases, HIV infected TB cases who failed treatment and retreatment smear-positive cases on follow-up.[6,7]

Drug-resistance TB (DR-TB) treatment centers were set-up under the RNTCP for provision of these high quality DST services which were free of cost. As of 2017, 147 DR-TB centers have been established across India with one for approximately every 10 million population.[7] The state of Delhi also adopted the PMDT in 2009 and set-up four DRTB centers, three of which were selected for the present study. The information recorded on treatment cards of all the drug-resistant TB patients registered and initiated on treatment between Jan 2009 to December 2014 in these DRTB centers was retrieved. Treatment outcomes were ascertained until the fourth quarter of 2016. Information pertaining to drug sensitivity testing was also retrieved from the registers maintained in the respective centers. Data collection process for this study was undertaken in 2017.

For the purposes of this study, a case of multidrug-resistant tuberculosis (MDR-TB) is defined as sputum/isolate in which the culture is positive for *Mycobacterium tuberculosis* and is found to have in vitro resistance to isoniazid and rifampicin with or without resistance to other anti-TB drugs based on additional Drug Sensitivity Test (DST) results. Furthermore, an extensively drug-resistant tuberculosis (XDR-TB) case is defined as an MDR-TB case whose recovered *M. tuberculosis* isolate is resistant to at least isoniazid, rifampicin, plus either a fluoroquinolone (Ofloxacin) or a second-line injectable anti-TB drug (kanamycin). Detailed resistance pattern was recorded for each first and second line anti-TB drugs during the period of observation.

Ethical waiver for the study was obtained from the Institutional Ethics Committee, Maulana Azad Medical College & associated Lok Nayak Hospital, New Delhi.

Statistical analysis was conducted using IBM SPSS Version 17 (Chicago, IL). Data was expressed in frequency and proportions. The trends and patterns of drug sensitivity patterns in the MDR-TB cases and their associated treatment outcomes has been reported.

3. Results

A cumulative total of 2958 MDR-TB cases were registered for initiation of therapy in the three drug-resistant TB (DRTB) treatment centers during the period from Jan 2009 to December 2014. The median value of time interval between culture test result and initiation of treatment was 82 days and it ranged between 0 days and 845 days (Table 1).

Among the MDR-TB cases initiated on treatment, almost all (99.1%) were resistant to Rifampicin and most (92.2%) were resistant to Isoniazid. A high burden of drug resistance was also observed against the other first-line anti-TB drugs. A total of 857 cases were evaluated for Streptomycin and 814 for Ethambutol resistance of which 600 (70%) and 327 (40.1%) were found resistant respectively.

Resistance to the second-line anti-TB drugs Ofloxacin and Kanamycin was assessed among 426 and 408 cases of which resistance was observed in 181 (42.4%) and 49 (12%) cases respectively (Table 2).

The proportions of cases with resistance to Streptomycin and Ethambutol increased over time. Among second-line drugs, resistance to Ofloxacin and Kanamycin increased from 2009 to 2013 with a subsequent reduction observed in 2014 (Table 3).

Table 4 depicts data on the additional resistance recorded among the MDR-TB patients. Resistance to four of the first-

Table 1 – Time interval for initiation of treatment in MDR-TB cases.

Time interval between culture results and treatment initiation (N = 1269)[a,b]	
Median	82 days
Minimum	0 days
Maximum	845 days
Percentiles	
25th	30 days
50th	82 days
75th	139 days

[a] 260 patient initiated on treatment before culture result (excluded).
[b] The data for 1429 cases could not be accessed.

Table 2 – Drug resistance pattern (first and second-line anti-TB drugs) for MDR-TB cases initiated on treatment.

	Resistance N (%)	Susceptible N (%)	Total (N)
Streptomycin(S)	600 (70)	257 (29.9)	857
Isoniazid(H)	2388 (92.2)	200 (7.7)	2588
Rifampicin(R)	2846 (99.8)	5 (0.1)	2851
Ethambutol(E)	327 (40.1)	487 (57.9)	814
Second line drug			
Ofloxacin (Ofx)	181 (42.4)	245 (57.5)	426
Kanamycin(K)	49 (12)	359 (87.9)	408

line anti-TB drugs (Rifampicin, Isoniazid, Streptomycin and Ethambutol) was present in nearly one-third (35.6%) of the tested cases. XDR-TB was seen in 24 cases and Pre-XDR/TB in 124 cases.

The MDR-TB cases resistant to only the first-line anti-TB drugs were more likely to show favorable outcome at end of treatment. Among MDR-TB cases who were only resistant to rifampicin and isoniazid, more than half (52.5%) showed favorable treatment outcome while in those cases who were resistant to four of the first-line anti-TB drugs (54.9%) showed favorable treatment outcome. However, most pre XDR-TB (73.7%) and XDR-TB (87.5%) cases had adverse treatment outcomes (Table 5).

4. Discussion

The present study conducted in three drug-resistant TB treatment centers in Delhi analyzed the trends in drug sensitivity pattern in 2589 MDR-TB cases reported and initiated on treatment from 2009 to 2014. Second line (Kanamycin, Ofloxacin) drug sensitivity tests (DST) were conducted in less than one-fifth of the MDR-TB cases. The DST showed that individual drug resistance was high for streptomycin (70%), Ethambutol (40.1%) and Ofloxacin (42.4%) but was relatively lower for Kanamycin (12%) among the tested cases. These findings were consistent with the figures reported in another study that evaluated drug susceptibility profiles for MDR-TB cases from the Eastern European countries of Estonia, Latvia and Romania.[8]

Our study observed a gradually increasing trend of cases with resistance to streptomycin and ethambutol. This finding is similar to that reported in an Iranian study which showed significantly increasing resistance to all of the first-line anti-TB drugs in retreatment TB cases.[9] However, despite the similar trends, a higher proportion of resistant cases was seen in the present study which is a concern due to the much greater burden of TB in India.[10]

A significant proportion of MDR-TB cases were also resistant to the second-line drugs (Ofloxacin, Kanamycin) which comprise the mainstay of treatment for MDR-TB was found in the present study. Growing resistance to second-line anti-TB drugs would increase treatment costs, worsen treatment outcomes and increase TB-related mortality. Similar findings have been previously reported from Taiwan where an increase in the rates of resistance to fluoroquinolones including Ciprofloxacin, Ofloxacin, and Levofloxacin was seen in the MDR-TB cases. Furthermore, the researchers found resistance rates (20%) were higher among strains isolated from 1998 to 2003 as compared to those obtained during 1995–1997 (7.7%).[11]

Our study analyzed the drug resistance to the different combination of drugs used in treating MDR-TB. Resistance to the four first-line anti-TB drugs (Rifampicin, Isoniazid, Streptomycin and Ethambutol) was present in 35.9% cases. A retrospective record based study among 788 MDR-TB cases from three states of India reported 42% resistance against all the first line anti-TB drugs.[12] However, a cross-sectional study in Ahmedabad city in Gujarat, India reported resistance against these four first-line anti-TB drugs in 70% cases (N = 81).[13] Furthermore, our study found resistance to second-line drugs – ofloxacin and kanamycin apart from rifampicin and isoniazid which meets the criteria of XDR TB to be present in 5.9% cases while Pre XDR-TB was seen in 30.5% cases. A study in Mumbai reported a higher proportion of cases with XDR-TB (9.7%) and also Pre XDR-TB (57%).[14] These findings suggest that drug sensitivity pattern in MDR-TB cases show significant variability across India.

In the present study, favorable treatment outcomes ranged from 52.5% to 56.9% in cases resistant to only first-line anti-TB drugs. The adverse outcomes were high among those cases who showed resistance to second-line anti-TB drugs. A meta-analysis conducted by Kibret et al which included 14 observational studies reported a pooled cure rate of 55.6% in 5047 MDR-TB cases treated with DOTS-Plus which is similar to our study.[15] Another study conducted in Mumbai, India reported a lower cure rate (48.4%) in MDR-TB cases initiated on treatment.[16]

It is well established that the delay in initiating treatment prolongs the time that patients carry the TB bacteria and allows the disease to progress with worsening of treatment outcomes.[17] The apparently delayed initiation of treatment in MDR-TB cases from the time of availability of culture results observed in the present study is a cause for concern. Reasons for delay in the treatment may be attributed to the patient's poor knowledge about TB, financial burden and poor accessibility to TB care. Further, social stigma and lack of social support in patients hinder timely treatment seeking behavior in TB patients.

A limitation of the present study is that less than one-fifth of the MDR-TB cases were evaluated for second-line DST. However, according to the National Strategic Plan for TB

Table 3 – Resistance profile (first and second-line anti-TB drugs) of MDR-TB cases initiated on treatment from inception (2009) till 2014.

Drugs	2009	2010	2011	2012	2013	2014
Streptomycin	(N = 174)	(N = 228)	(N = 256)	(N = 103)	(N = 70)	(N = 26)
Resistance	103 (59.1)	172 (75.4)	171 (66.7)	72 (69.9)	57 (81.4)	25 (96.1)
Isoniazid	(N = 175)	N=(231)	N=(308)	N=(797)	N=(655)	N=(422)
Resistance	175 (100)	225 (97.4)	296 (96.1)	690 (86.5)	616 (94)	386 (91.4)
Rifampicin	(N = 175)	(N = 231)	(N = 306)	(N = 831)	(N = 705)	(N = 603)
Resistance	175 (100)	231 (100)	305 (99.6)	831 (100)	703 (99.7)	601 (99.6)
Ethambutol	(N = 172)	(N = 224)	(N = 249)	(N = 94)	(N = 55)	(N = 20)
Resistance	43 (25)	96 (42.8)	96 (38.5)	39 (41.4)	40 (72.7)	13 (65)
Second line drugs						
Ofloxacin	(N = 6)	(N = 7)	(N = 16)	(N = 23)	(N = 58)	(N = 316)
Resistance	1 (16.6)	2 (28.5)	9 (56.2)	12 (52.1)	41 (70.6)	116 (36.7)
Kanamycin	(N = 3)	(N = 7)	(N = 14)	(N = 16)	(N = 57)	(N = 311)
Resistance	0	1 (14.2)	1 (7.1)	4 (25)	13 (22.8)	30 (9.6)

Table 4 – Additional resistance to first and second line anti-TB drugs among MDR-TB patients in Delhi, 2009–2014.

Number of additional anti-TB drugs to which MDR-TB patients were resistant	Resistant (N = 2958)	%	Total
Rifampicin(R)+ Isoniazid(H)	2358	91.9	2564
Rifampicin(R)+ Isoniazid(H)+ Streptomycin(S)	579	69.0	839
Rifampicin(R)+ Isoniazid(H)+ Ethambutol(E)	319	39.4	809
Rifampicin (R)+ Isoniazid(H) + Ethambutol(E) + Streptomycin(S)	289	35.9	803
Second line drug(s)			
Rifampicin (R) or Isoniazid(H) + Ofloxacin (Ofx) + Kanamycin(K)	40	9.8	407
Rifampicin (R) + Isoniazid(H) + Ofloxacin (Ofx) + Kanamycin(K) (XDR-TB)	24	5.9	407
Rifampicin (R) + Isoniazid(H) + (Ofloxacin (Ofx)/Kanamycin(K)) (Pre-XDR/TB)	124	30.5	407

elimination in India (2017–2025), all the Rifampicin Resistant and MDR-TB patients will be subjected to baseline DST for Kanamycin and Levofloxacin[18].

In conclusion, the present study observed growing resistance against first and second-line anti-TB drugs in MDR-TB cases treated in Delhi from 2009 to 2014. Adverse treatment outcomes were found in nearly half of the MDR-TB in whom resistance was restricted to first-line anti-TB drugs and most cases who showed additional resistance to second-line anti-TB drugs. These findings signifies the need to accelerate steps towards the prevention of emergence of drug-resistant TB strains and improvement of outcomes which was much lower than the WHO target of 75–90% treatment success rate.[19] Moreover, the present data advocates the need for developing specific interventions to improve linkage of MDR-TB diagnosis to initiation of treatment since earlier treatment initiation will reduce the duration of infectiousness and aid in interrupting the chain of transmission. Hence, treatment for MDR-TB should clearly be initiated at the earliest opportunity after diagnosis which was delayed as per our study findings. Future studies should improve focus upon improving data collection and reporting of patient outcomes by time to initial diagnosis and treatment initiation to provide further insight into this less understood phenomenon.

Table 5 – Resistance pattern and treatment outcome among MDR-TB cases.

Number of additional anti-TB drugs to which MDR-TB patients were resistant	Favorable outcome N (%)	Adverse outcome N (%)	Other N (%)	Resistant total N (%)
Rifampicin(R)[b] Isoniazid(H)	1146 (52.5)	950 (43.5)	88 (4.0)	2184[a]
Rifampicin(R)[b] Isoniazid(H)[b] Streptomycin(S)	309 (56.9)	218 (40.1)	16 (2.9)	543[b]
Rifampicin(R)[b] Isoniazid(H)[b] Ethambutol(E)	161 (54.6)	121 (41.0)	13 (4.4)	295[c]
Rifampicin(R)[b] Isoniazid(H)[b] Ethambutol(E)[b] Streptomycin(S)	146 (54.7)	110 (41.2)	11 (4.1)	267[d]
Second line anti-TB drugs				
Rifampicin(R)[b] Isoniazid(H)[b]Ofloxacin (Ofx)[b] Kanamycin(K) (XDR-TB)	3 (12.5)	21 (87.5)	0 (0)	24
Rifampicin(R)[b] Isoniazid(H)[b](Ofloxacin (Ofx)/Kanamycin(K)) (Pre-XDR/TB)	26 (26.3)	68 (68.7)	5 (5.1)	99[e]

[a] 174 outcome not available.
[b] 36 Outcome not available.
[c] 24 Outcome not available.
[d] 22 Outcome not available.
[e] 25 outcome not available.

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Conflicts of interest

All authors have none to declare.

Author contributions

Dr. Nandini Sharma, Dr Shivani Chandra contributed to the design, concepts, literature search, data acquisition and data analysis. Dr Nandini Sharma also contributed to the literature search, statistical analysis and manuscript preparation.

Dr Neeta Singla contributed to data acquisition, data analysis and manuscript preparation.

Dr Ashwini Khanna contributed to the design, concepts and data acquisition.

Dr. Saurav Basu contributed to literature search, data analysis, statistical analysis and manuscript preparation.

Dr. K K Chopra contributed towards concepts.

Dr. Charu Kohli contributed to the data interpretation and manuscript preparation.

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Original article

Oxidative stress and its impact on mitochondrial DNA in pulmonary tuberculosis patients- a pilot study

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ABSTRACT

Background: Pulmonary tuberculosis (PTB) remains a major cause of morbidity and mortality all around the world. Recent studies have pointed out increased oxidative stress and also DNA damage in peripheral blood in PTB. Till date, to the best of our knowledge, no study has so far been conducted to show the mitochondrial DNA (mtDNA) deletions mapping in PTB patients. Therefore we performed the present study with the aim to investigate oxidative stress parameters along with mtDNA damage in newly diagnosed untreated PTB patients.

Material and methods: This is a prospective study carried out in Mahatma Gandhi Institute of Medical Sciences, Sevagram,Wardha, Maharashtra during september 2017 to september 2018.Thirty newly diagnosed untreated PTB patients and thirty age matched healthy controls were enrolled in the present study. Analysis of Oxidative stress parameters such as nitric oxide (NO) and malondialdehyde (MDA) were done by calorimetric methods. Assessment of mitochondrial DNA damage was carried out by mtDNA deletions mapping using primer shift long range polymerase chain reaction technique.

Results: There was significant increase in levels of oxidative stress parameters, nitric oxide and malondialdehyde, in PTB patients compared to controls ($p < 0.01$). Generally there are two common deletion sites of "13 bp direct repeats" (ACCTCCCTCACCA) in mtDNA. One at the junction sites from bp 8470 to 8482 bp and another from bp 13447 to 13460 bp which make mtDNA more prone for 4977bp deletion. Out of thirty cases of PTB, two cases showed mtDNA damage in the form of mtDNA deletion of 4977bp. There was no mtDNA deletion in any control which can be attributed to continuous generation of oxidative stress.

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Conclusion: This pilot study has been able to demonstrate that compared to controls, in newly diagnosed pulmonary tuberculosis patients some mtDNA damage did occur and was probably due to continuous generation of oxidative stress in tuberculous patients. However, sample size is too small to draw any conclusions but definitely a more comprehensive study, by recruiting more number of pulmonary tuberculosis patients is warranted to establish correlation between oxidative stress and mtDNA damage in PTB.

1. Introduction

Tuberculosis (TB) is a well known infectious disease which remains an issue of high morbidity and mortality worldwide. The causative agent of tuberculosis is *Mycobacterium tuberculosis*.[1] When it affects lungs it is referred as pulmonary tuberculosis (PTB) but can affect other organs when it is referred to as extra pulmonary tuberculosis (EPTB).[2] *Mycobacterium tuberculosis* is an intracellular pathogen that grows and replicates in the host macrophages. As a result of this, there is production of reactive oxygen and reactive nitrogen intermediates which remain the major response of macrophages to tuberculous infection. Such reactive oxygen and nitrogen intermediates are highly toxic and their interaction with the body cells even leads to deterioration of normal cell function.[3] Routinely during cellular metabolic processes, these reactive molecules are generated but are neutralized by the antioxidants existing in our body. But in tuberculosis, generation of these reactive molecules exceeds the neutralizing capacity of antioxidants due to their overproduction.[3,4] Increased levels of reactive molecules damage important biological molecules in cells including DNA.[5] Previous studies revealed that increased levels of reactive oxygen species (ROS) with depletion of antioxidants, ultimately create oxidative stress in pulmonary tuberculosis.[4,6] Several past researches have implicated the role of oxidative stress i.e. increased levels of reactive molecules in the development as well as prognosis of pulmonary tuberculosis.[1,7] There are a few studies which have reported the evidence of DNA damage in tuberculosis,[8,9] but little is known about the level of mitochondrial DNA damage in tuberculosis which is about 10–100 times more labile for mutations as compared to nuclear DNA.[10,11] Oxidative stress creates impairment in electron transport chain due to excessive amount of free radicals which leads to large scale deletions in mtDNA.[12,13] The molecular genetic analysis of mtDNA deletion in pulmonary tuberculosis patients may be very helpful in determining the prognosis in the patient. Till date, to the best of our knowledge, no study on mitochondrial DNA deletions mapping has so far been reported. Our intention for this prospective study was therefore to assess the levels of oxidative stress markers and its correlation with mitochondrial DNA damage in peripheral blood lymphocytes in newly diagnosed untreated pulmonary tuberculosis patients.

2. Material and methods

2.1. Patient selection criteria

The study comprised of two groups:

I. Thirty newly diagnosed untreated pulmonary tuberculosis patients of age group between 18 and 50 years.
II. Thirty age matched and sex matched healthy controls.

Subjects of either sex with age ranging from 18 to 50 years were included in the study. The diagnosis of pulmonary tuberculosis was made on the basis of clinical symptoms and bacteriologically positive sputum for *Mycobacterium tuberculosis* by smear and culture.[14] Written consent was taken from all the enrolled subjects after informing them about the study protocol. The genetic material which we had collected was used for the present study purpose only. Institutional Ethics Committee's permission for the present study was obtained.

2.2. Exclusion criteria

Subjects with coexistence of other diseases such as other respiratory disorders, diabetes mellitus, coronary heart disease, hypertension, liver diseases, human immunodeficiency virus infection and cancer were excluded from the study. Other exclusion criteria were age <18 years and >50 years, smoking habits, alcohol consumption. Additional exclusion criteria for women was pregnancy or lactation.

2.3. Blood DNA extraction

We have collected venous blood sample (total 5 ml) from all the thirty newly diagnosed untreated pulmonary tuberculosis patients as well as thirty healthy controls in EDTA containing bulb as well as plain bulb (2.5 ml each) after following the above mentioned inclusion and exclusion criterion. Total DNA (Nuclear & Mitochondria) of human peripheral blood lymphocytes was extracted from all the study subjects according to the standard protocol.[15] RBC lysis buffer was used for separation of lymphocytes from whole peripheral blood. Lymphocytes were incubated overnight at 56 °C in a lysis buffer containing 250 μl Sodium chloride EDTA buffer (SE buffer), 20μl proteinase K and 30 μl of 10% Sodium dodecyl sulphate (SDS). After digestion, the lysate was extracted with phenol, followed by phenol/chloroform/isoamyl alcohol (25:24:1),

chloroform/isoamyl alcohol (24:1) and 30 μl of 3M sodium acetate (pH5.6) with chilled absolute alcohol. Finally, precipitated DNA was treated with 70% ethanol (v/v), the DNA pellet was dried in hot air oven for 30 minutes and at the end DNA was dissolved in 100 μl Tris–EDTA buffer, pH 8.3 for 5–6 hours in hot water bath at 55 °C. DNA (mtDNA) sample was used for further analysis.

2.4. Long range polymerase chain reaction

A specific segment of mtDNA (>5 kb) was amplified from 100 ng of mtDNA of pulmonary tuberculosis patients in a 50 μl reaction mixture containing 200 μM of each dNTP, 1μM each of forward and reverse primers, 1IU of Taq DNA Polymerase, 50 mM KCL, 2 mM $Mgcl_2$, 25 mM Tris [hydromethyl] methyl-3-aminopropanesulphonic acid (TAPS), 1mM β-mercaptoethnol, and 10 mM Tris–HCl, pH 8.3.[16]PCR was carried out for 30 cycles in a DNA thermal cycler using the std. PCR conditions of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and primer extension at 72 °C for 1 min after the required number of cycles, an additional extension step at 72 °C for 3 min was performed. PCR products were checked on 1.5% agarose gel using 1x TBE buffer (89.2 mM Tris–HCL, 89mM Boric acid, 1.2 mM EDTA pH 8) at 150 volts for 1.5 hrs.

2.5. Assessment of oxidative stress markers

Nitric oxide (NO) was measured in plasma samples using Griess's reagent method.[17] Griess reagent consists of 1% sulfanilamide solution in 2.5% phosphoric acid and 0.1% napthylethylene diamine dihydrochloride (NED) solution. It was subjected to equilibrate at room temperature. Plasma sample, 50 μl, was taken in a tube. To this, 450 μl of distilled water and 500 μl of Griess reagent were added. Optical density was measured spectro-photometrically at 540nm against the blank containing distilled water and Griess reagent.

Estimation of Malondialdehyde (MDA) was done in plasma by modified TCA TBA method of Stater et al.[18] In the test tube 0.5 ml of plasma was taken. To this, 3ml of 10% trichloroacetic acid (TCA) was added. It was mixed properly and allowed to remain at room temperature for 10 minutes. It was subsequently centrifuged at 5000 rpm for 15 minutes. 1.5 ml of 0.67% thiobarbituric acid (TBA) was added to 2ml of supernatant fluid from centrifuged sample. After this, sample was kept in the boiling water bath for 10 minutes which was then cooled under tap water. Colour intensity was measured spectrophotometrically at 530nm.

2.6. Statistical analysis

The values of oxidative stress parameters were expressed as Mean ± SD. Student 't' test was used for comparative analysis of data between case and control groups. Statistical analysis was done using the Epi Info software.

3. Results

We have collected the venous blood samples from thirty newly diagnosed bacteriologically positive pulmonary tuberculosis patients (PTB) as well as thirty healthy controls in the age group ranging from 18 to 50 years.

3.1. Assessment of markers of oxidative stress

We have assessed the levels of nitric oxide (NO) and malondialdhyde (MDA), markers of oxidative stress, in all sixty participants (30 pulmonary tuberculosis cases and 30 controls). There was statistically significant increase in NO level in PTB patients as compared to healthy controls. The mean plasma level of NO was found to be 0.21 ± 0.06 M in healthy controls whereas 0.42 ± 0.09 M in PTB patients ($t = 10.88, p < 0.01$) (Table 1). Most of the individuals belonging to control group had NO level ranging from 0.15 to 0.30 M whereas NO level ranges from 0.30 to 0.60 M in maximum number of PTB cases (Fig. 1). In PTB patients, significantly increased levels of MDA were observed when compared with healthy controls. The mean plasma levels of MDA was found to be 3.81 ± 0.56 nmol/ml in controls whereas 7.55 ± 1.05 nmol/ml in PTB patients ($t = 17.14$, $p < 0.01$) (Table 1). Most of the individuals belonging to control group had MDA level ranging from 3.0 to 4.5nmol/ml whereas it ranges from 6.0 to 9.0nmol/ml in maximum number of PTB cases (Fig. 2).

3.2. Assessment of mtDNA deletion

The whole DNA (nuclear and mitochondrial) was extracted from EDTA containing peripheral blood lymphocytes from all the enrolled pulmonary tuberculosis (PTB) patients and healthy controls. Initially we used primer set L1-H1 for confirmation of mtDNA in the extracted DNA samples of PTB and control subjects (Table 2). We used MT2-MT4 primer set for screening of 4977 bp mtDNA deletion in all sixty participants (30 PTB cases and 30 controls). Out of 30 pulmonary tuberculosis patients, 2 (6.66%) patients showed deletion of 4977 bp. In these two cases, 5283bp band was the full length normal PCR product from the wild type mtDNA and

Table 1 – Comparison of oxidative stress parameters between pulmonary tuberculosis patients and controls.

Oxidative stress Parameters	Control (Mean ± SD)	Pulmonary tuberculosis patients (Mean ± SD)	t-value	p-value
Nitric oxide (NO)	0.21 ± 0.06 M	0.42 ± 0.09 M	10.88	p < 0.01,S
Malondialdhyde (MDA)	3.81 ± 0.56 nmol/ml	7.55 ± 1.05 nmol/ml	17.14	p < 0.01,S

S-significant, SD-standard deviation

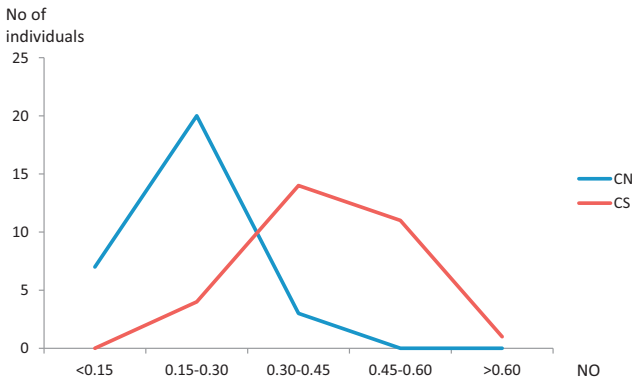

Fig. 1 – Line diagram showing Nitric oxide levels among pulmonary tuberculosis cases (CS) and controls (CN).

approximately 306bp band was generated from 4977 bp deleted mtDNA whereas no deletion of 4977bp was observed in any of the control group showing full length normal PCR product of 5283 bp (Figs. 3 and 4).

Furthermore these deletions were confirmed by primer shift PCR using another set of primers as MT1-MT3 (Table 2). The 5356bp band was the full length normal PCR product from the wild type mtDNA and approximately 379bp band was found from 4977 bp deleted mtDNA in two out of thirty pulmonary tuberculosis patients suggesting large scale 4977 bp deletion of mtDNA. After successful PCR reaction, on agarose gel electrophoresis appearance of two bands (5356bp and 379bp) was due to heteroplasmic mtDNA which is a combination of wild type as well as deleted mtDNA. In mtDNA two "13 bp direct repeats" (ACCTCCCTCACCA) are present at the junction sites from bp 8470 to 8482 and from bp 13447 to 13460 bp. It increases susceptibity of mtDNA for 4977bp deletion due to continuous generation of oxidative stress in their environment.

When a comparison was made among PTB patients, out of thirty cases, two cases showing mtDNA deletion exhibited remarkably increased levels of oxidative stress parameters compared to other PTB patients having no mtDNA deletion.

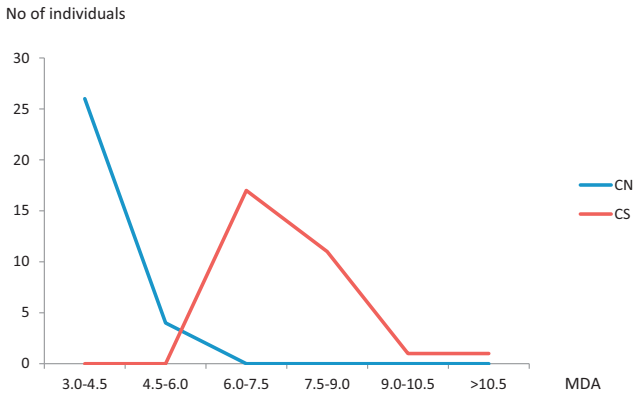

Fig. 2 – Line diagram showing malondialdehyde levels among pulmonary tuberculosis cases (CS) and controls (CN).

4. Discussion

In PTB, there occurs overproduction of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) as a major immune response of host macrophages against the *Mycobacterium tuberculosis* bacillus that grows and replicates in it.[7] Production of huge amount of ROS and RNI kills *Mycobacterium tuberculosis* bacillus. In addition, these ROS lead to membrane lipid peroxidation (LP)[19,20] which is one of the general mechanisms of tissue damage by free radicals resulting in pathological events.[7] One of the major aldehyde byproducts of lipid peroxidation is malondialdehyde (MDA) which accumulates in the blood circulation as well as in tissues under oxidative stress conditions[21] and thus is a good indicator of oxidative stress.

Oxidative stress can be evaluated not only by measuring the levels of lipid peroxidation products (MDA and Thiobabituric acid reducing substances TBARS), nitric oxide (NO) but also by assessment of enzymatic antioxidants (Superoxide dismutase SOD, Catalase CAT) and non-enzymatic antioxidants (Vitamin C and E).[22–24]

In the present study, oxidative stress was evident as indicated by significantly increased levels of MDA in pulmonary tuberculosis patients in comparison to healthy controls. Kulkarni et al (2015) studied serum MDA in PTB patients where they observed significantly increased MDA levels in PTB patients as compared to controls.[25] Kandukuri et al reported significantly increased levels of MDA as an indicator of progression of TB disease. They also commented that estimation of MDA is an important biochemical marker in assessing the progression of TB.[26] Earlier studies also reported enhanced lipid peroxidation product MDA in PTB patients.[4,7] There occurs gradual decrease in MDA levels with clinical improvement of PTB patients with anti-tuberculosis therapy.[7]

A major host defense against *Mycobacterium tuberculosis* is nitric oxide (NO) which is produced by activated macrophages.[27] Thus NO is an important oxidative stress marker to be studied in tuberculosis infection. In the present study, levels of NO were significantly increased in PTB patients with respect to age matched healthy controls ($p < 0.01$). Mohod et al (2011) reported that NO, being an important reactive nitrogen species, has strong implication in TB and further commented upon significantly elevated levels of NO in all PTB patients with varying bacillary load compared to controls.[28] Recent study also demonstrated the findings which coincided with Mohod et al.[1] Active tuberculosis has been reported to elevate

Table 2 – Oligonucleotide primers i) L1-H1 used to determine mtDNA ii) MT2-MT4 used for detection of 4977 bp mtDNA deletions and iii) MT1-MT3 used to assess 4977 bp deletions in pulmonary tuberculosis patients.

Sr. No.	Primer	F or R	Location	PCR product
1.	L-1	F	3304–3323bp	413bp
	H-1	R	3717–3698bp	
2.	MT1	F	8224–8247bp	5356bp
	MT3	R	13580–13551bp	
3.	MT2	F	8286–8304 bp	5283bp
	MT4	R	13569–13548 bp	

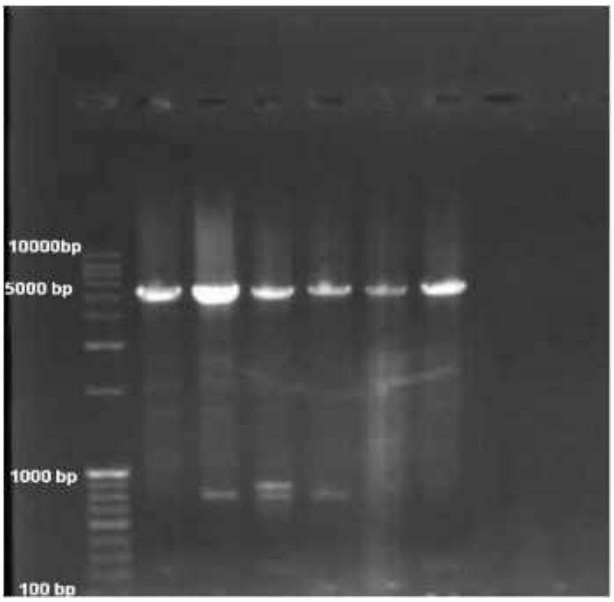

Fig. 3 – Agarose gel electrophoresis analysis showing full length amplified Polymerase chain reaction (PCR) product of 5285bp in healthy controls (using primer MT2-MT4).

urinary levels of the NO metabolites, nitrite and nitrate, and these levels decreased with anti-TB treatment in tuberculosis patients.[29] They explained that endogenous generation of NO is a defense mechanism against *Mycobacterium tuberculosis*.[30,31] The earlier study also suggested significant increase in NO as well as MDA levels in PTB patients. As the severity of TB increases from cat I to cat III, higher oxidative stress is generated to resist the increasing bacillary load of *Mycobacterium tuberculosis* by causing lipid peroxidation of polyunsaturated fatty acids present in the cell membrane of pathogenic microorganism.[24]

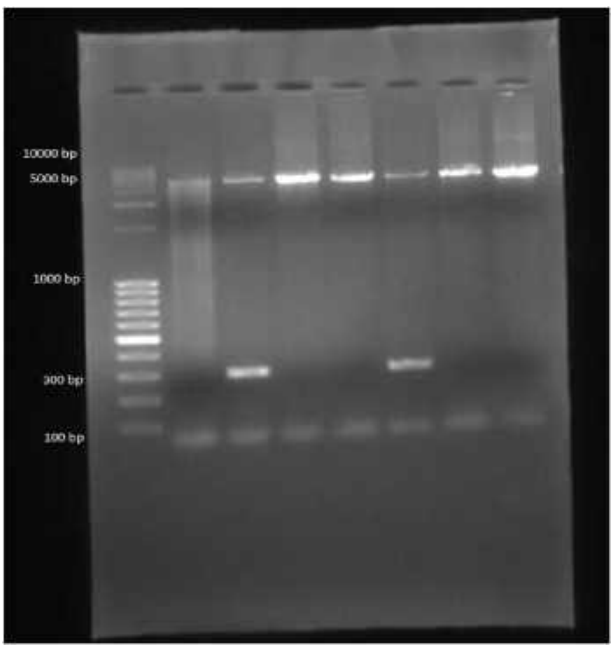

Fig. 4 – Agarose gel electrophoresis analysis showing 306 bp band as well as full length amplified Polymerase chain reaction (PCR) product of 5283 bp in pulmonary tuberculosis patients (using primer MT2-MT4).

Pulmonary tuberculosis is associated with malnutrition due to low dietary supplementation of anti-oxidants which further enforces the body to use the available anti-oxidants to neutralize the increased ROS generated in TB. Thus creating a pathogenic loop giving rise to further enhancement of oxidative stress.[7] Presence of oxidative stress for longer duration in the lung cells of the diseased serves as a source of free radicals that have damaging effect on DNA of pulmonary and circulating cells,[32–34] thus contributing to pathogenesis of lung diseases.

Previous studies in male infertility have stated that one of the important indicators of mtDNA damage is a 4977bp deletion which is known as common deletions in mtDNA.[35,36]

In the present study with new PTB patients, we have also found mitochondrial DNA damage in the form of 4977bp deletion in two out of thirty pulmonary tuberculosis cases. There are two common deletion sites of "13 bp direct repeats" (ACCTCCCTCACCA) in mtDNA. One at the junction sites from bp 8470 to 8482 and another from bp 13447 to 13460 bp which increased the susceptibity of mtDNA for 4977bp deletion.[35] Studies on infertile males also confirmed the role of two direct repeats of 13 bp leading to 4977 bp mtDNA deletions in sperms of infertile male patients.[35,36] Buccal micronucleus cytome assay (BMCyt) is also used as an indicator of DNA damage in tuberculous patients leading to cellular frequency consistent with problems in cytokinesis, DNA repair and DNA damage in TB patients.[37] There occurred micronucleus formation in damaged cells as a result of non-repaired or misrepaired DNA damage due to oxidative stress in TB patients. When level of damage is very high, these damaged cells are expelled out by apoptosis.[38] Narmadha et al (2014) investigated DNA damage by comet assay of white blood cells in PTB patients. They observed very significant increase in percentage of DNA in tail which was nothing but damaged DNA leached out of the cells in newly diagnosed TB patients when compared with control group.[9]

In another study on PTB patients under anti-tuberculous treatment, increased DNA damage has been reported in peripheral blood mononuclear cells in patients.[39] As newly diagnosed PTB patients recruited in our study were not on anti-tuberculous medication, thus increased oxidative stress may be due to higher bacterial stimulation which coincided with the finding of Bhargava et al.[40]

There are several in vitro cell culture studies showing DNA fragmentation and induction of human alveolar macrophages apoptosis by *Mycobacterium tuberculosis* infection.[41,42] Increased peripheral DNA damage correlating significantly with oxidative stress has been shown in tuberculous pleurisy patients.[43]

It has been suggested that oxidative stress and DNA damage are increased in pulmonary tuberculosis patients. Raised oxidative stress associated DNA damage can be considered as an important pathogenetic mechanism involved in the risk of developing lung cancer in PTB.[8] *Mycobacterium tuberculosis* could cause genetic alterations thereby increasing susceptibility to development of cancer.[44] Mitochondrial DNA is about 10 times more prone to get damaged than nuclear DNA due to

increased oxidative stress.[45] Over the years mitochondria is a power house of cell. Study of mtDNA in PTB due to increased oxidative stress remains neglected in the field of research. It has been reported that gastrointestinal TB could increase mitochondrial oxidative stress and lower mtDNA copy number.[41] Nuclear DNA damage and decreased activities of mitochondrial respiratory chain enzymes has also been observed in gastrointestinal TB.[40] In the present study, only 2 out of 30 PTB patients showed mtDNA damage in the form of 4977bp deletion. As these were newly detected patients it is reasonable to presume that this may manifest more in patients with longer duration of disease where oxidative stress would be even more. Oxidative stress associated DNA damage in PTB patients depict the role of supplementation of antioxidants as an adjuvant therapy along with anti-tuberculosis drugs while treating PTB patients to have good prognosis and better future outcome of such patients.[6,46] However, further large scale prospective studies are needed to clarify and establish the association between mtDNA damage and oxidative stress in PTB covering various aspects of treatment like responders and non-responders as well as role of administration of antioxidants as an adjuvant therapy in treating such patients.

5. Conclusion

It is concluded that significant increase in oxidative stress markers, NO and MDA, exists in the newly diagnosed untreated pulmonary tuberculosis patients compared to healthy controls. Furthermore this preliminary study has been able to demonstrate that some mtDNA damage did occur in newly diagnosed PTB patients. Present study was conducted on newly diagnosed PTB patients who might not have been exposed to increased oxidative stress for longer interval of time.

Thus we recommend a more comprehensive study involving larger number of pulmonary tuberculous patients from different categories of diseases should be studied in order to further strengthen the above findings to establish correlation between mtDNA damage and oxidative stress in PTB.

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.2019.03.001.

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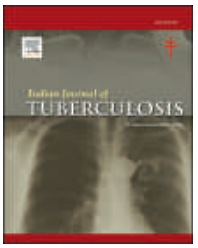

Original article

Role of microbiological tests in diagnosis of genital tuberculosis of women with infertility: A view

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ABSTRACT

Objective: India is a country sharing one fourth of the global incidence of tuberculosis. It is much easier to diagnose pulmonary cases, but challenges are with extrapulmonary cases. Genital tuberculosis is considered as an important cause of infertility in young females in India and difficult to diagnose. It requires incorporation of different modalities that should correctly, timely and rapidly diagnose the case.

Methods: This study was conducted retrospectively for a period of 12 months on 438 endometrial samples from females with history of infertility. Three modalities namely Ziehl-Neelsen staining, Automated liquid culture and Nucleic acid amplification technique (TB-PCR) were compared and their sensitivity in diagnosis of genital tuberculosis was ascertained.

Results: Out of 438 samples, 18 samples were found positive with at least one modality. TB-PCR positivity was 3.6% (16 cases) in comparison to culture where positivity was 1.59% (7 cases). Five samples were found culture and TB-PCR positive and only one sample was positive by all three diagnostic tests.

Conclusion: Infertility in young female per se is usually heart breaking and distressing. Therefore, it is essential to diagnose and treat the cases of genital tuberculosis before irreversible damage of tube may happen. Although, advancement in diagnostic field is there from microscopy to molecular method, but still diagnosis of genital tuberculosis is challenging. Correct diagnosis prevents young female from mental trauma and toxicity of anti-tuberculosis drugs given on suspicion in high prevalence country like India.

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1. Introduction

Tuberculosis was not only present in our ancient time but remains an unsolved problem till date.[1] According to the World Health Organization (WHO) global report 2014, tuberculosis (TB) is the deadliest communicable disease and a serious public concern worldwide that primirarily affects lungs but can be extra-pulmonary also.[2] Incidences are high in developing countries in comparison to the developed countries.

Extra-pulmonary tuberculosis (EPTB) can involve any part of the body, but most commonly affects lymph node, pleura, genitourinary tract, bones and joints, meninges, peritoneum and pericardium. Among these, genital tuberculosis is a cause for concern and emerging as a serious health issue.[3]

Estimated global prevalence of genital tuberculosis is about 8–10 million cases.[4] In India, 5–13% females are affected with preponderance in age group of 20–40 years.[5] Genital tuberculosis is mostly under diagnosed, overlooked and under reported because of its asymptomatic presentation and paucibacillary nature.[6]

The most common presentation of genital tuberculosis is infertility (43–74%), and others are pelvic pain, menstrual irregularities and dyspareunia.[7] Commonly affected sites are fallopian tube (95–100%), followed by endometrium (50–60%), ovaries (20–30%), cervix (5–15%), myometrium (2.5%) and vulva/vagina (1%). In India, infertility leads to a lot of mental and social distress in young females.[8]

Varied clinical presentations of genital tuberculosis give rise to diagnostic dilemma which are further confounded by diverse results in laproscopy, imaging studies, histopathology, culture and nucleic acid amplification techniques with varied diagnostic sensitivities and specificities.

Among these modalities, most common is Ziehl-Neelsen (ZN) smear microscopy, which is simple, inexpensive but has poor sensitivity (needs >10,000 bacilli/ml of sample). Mycobacterial culture, although considered as the gold standard usually takes 2–6 weeks for positive results, requires 10–100 bacilli/ml of sample and depends upon proper infrastructure and technical expertise.[4,9,10]

Different molecular techniques like nucleic acid amplification (NAA) have been developed. for early detection and identification of *Mycobacterium tuberculosis* (MTB) in clinical samples of pulmonary and extra-pulmonary tuberculosis. These techniques have high sensitivity and able to detect one to ten bacilli in various specimens [11,12]. These techniques are benefic in pauci-bacillary samples of extra-pulmonary cases.

Although there are multiple microbiological diagnostic modalities available, but all exhibit different advantages and disadvantages When we use these techniques in conjunction with each other, it increases the possibility of better diagnosis of tuberculosis. But, in case of genital tuberculosis, various modalities are being used on vague clinical suspicions and/or inadequate workup, it may lead to initiation of empirical Anti tubercular therapy. This may end up with doubtful therapeutic cure and emergence of side effects and drug toxicity.

Here in our study, we have compared three diagnostic modalities namely ZN staining, Automated liquid culture (MGIT) and Nucleic acid amplification technique (TB-PCR) to ascertain the sensitivity of these tests and their role in diagnosis of genital tuberculosis.

2. Materials and method

This 1 year study was done retrospectively from January to December 2016 on 438 endometrial specimens from females with history of infertility. The samples were received from different centers, associated with Oncquest Laboratory Ltd, New Delhi as per the laid down collection and transportation policy. Patient related information (like age, clinical history etc.) was gathered from test requisition forms (TRF). The TRF had very limited data on laparoscopic, hysteroscopic and histo-pathological findings and were therefore excluded from the study.

3. Inclusion criteria

Samples received with history of infertility.

Samples with requisition for all three tests.

4. Exclusion criteria

Sample received in formalin.

Samples with request of only culture or PCR.

5. Sample processing

Each sample was divided into two parts; one part was used for ZN microscopy and MGIT BACTEC 320 liquid culture in microbiology section and the second part sent for TB-PCR in the molecular section. All tests were performed on the same day in respective sections. For microscopy and liquid culture, a minimum of two ml of the sample was used and the rest was handed over to the concerned department for TB-PCR.

5.1. *Direct smear microscopy*

The sample was homogenized using a mortar and pestle. Smear was prepared and stained using conventional ZN staining method for detection acid fast bacilli (AFB). Smears showing red colored acid fast bacilli were taken as positive and those without any acid-fast bacilli, considered negative.[13]

5.2. *For culture*

Homogenized tissue sample was decontaminated using the N-acetyl-L cysteine-sodium hydroxide method (NALC-NaOH) and inoculated in Mycobacteria Growth Indicator Tube (MGIT) containing 4 mL of modified Middlebrook 7H9 Broth base and incubated in MGIT BACTEC 320 liquid culture system.[14] When the tubes were flagged positive by the system, ZN staining and culture on 5% sheep blood agar were performed from the tube directly to rule out any contamination as per the manufacturer's instructions. All tubes were checked till 42 days for positivity. From positive culture

tubes, Non-tuberculous mycobacteria (NTM) and *Mycobacterium tuberculosis* differentiation was done by rapid immunochromatography test kit using MPT 64 antigen according to the manufacturer's instructions (SD bioline, Standard Diagnostics.Inc.)

5.3. TB-PCR

Second part of the sample, sent to Molecular department was homogenized in a sterile container by homogenizer. Decontamination was done by N-acetyl L- Cysteine (NALC)–NaOH method and then centrifuge at 8000 rpm for 15 minutes as per the manufactures instructions (commercially available kit Viogene, Taiwan). All collected sediments were aliquoted into multiple vials for rechecking if needed and the remaining were processed for PCR within 24 hours or stored at − 20 ° C in a freezer until tested.

5.3.1. *Amplification of mycobacterium DNA*

DNA was extracted from specimens as per the manufacture's instruction (Viogene, Taiwan).

Real time PCR: A single tube Real time PCR was performed using the proprietary IS6110 specific primers and specific probes (Lyte star TB/NTM PCR Kit 2.0) targeting Mycobacterium tuberculosis complex and 16 S r DNA specific primers for Non-Tuberculosis Mycobacteria (NTM). The probes are labeled with fluorescent reporter and quencher dyes. Probes specific for MTBC and NTM DNA are labeled with the fluorophore FAM and HEX respectively. The probe specific for the internal control (IC) is labeled with the fluorophore Cy ^{5}. The test consists of two processes in a single tube assay. PCR amplification of target DNA and Internal Control simultaneously detection of PCR amplicons by fluorescent dye labeled probes.

6. Statistical analysis

Statistical analysis was done using MedCalc Version 16.8. The results of AFB stain, culture and TB-PCR was analyzed by Fisher's exact test. The Fisher's exact test static value for AFB stain and culture was 0.016 and 0.00001 for TB-PCR and culture. Both the results were found statistically significant at $P < 0.05$.

7. Results

Total 438 endometrial samples were processed for all three tests. Out of these, 18 samples were tested positive by at least one test modality. The results are as under:

Number of samples found positive in ZN smear microscopy: 01.

Number of samples found positive in culture: 07.

Number of samples found positive in TB PCR: 16.

Sensitivity, specificity, PPV and NPV of AFB stain and TBPCR with culture as a gold standard has been shown in Table:1.

Table 1 – Comparison of results from TB PCR and ZN smear microscopy with culture as gold standard.

Sample tested- 438		Culture		Sensitivity	Specificity	PPV	NPV	P value
		Positive	Negative					
AFB stain	Positive	1	0	14.29% (95%CI– 0.36% to 57.87%)	100% (95%CI– 99.15% to 100%)	100% (95%CI– 2.50% to 100.00%)	98.63% (95% CI– 97.04% to 99.49%)	0.016
	Negative	6	431					
TB PCR	Positive	5	11	71.43% (95%CI– 29.04% to 96.33%)	97.45% (95%CI– 95.48% to 98.72%)	31.25% (95% CI– 11.02% to 58.66%)	99.53% (95%CI– 98.30% to 99.94%)	0.00001
	Negative	2	420					

Samples AFB negative, liquid culture positive and TB PCR positive:5.

Samples AFB negative, culture negative & TB PCR positive:11.

Samples AFB negative, culture positive TB PCR negative:2.

Sample positive by all three methods:1.

Table:2 is showing the overall positivity along with positivity in positive samples.

Out of 16 TB PCR positive samples, 14 samples were Mycobacterium tuberculosis positive and two were Mycobacterium other than tuberculosis (MOTT). Among 7 cultures positive samples, four were MTB positive and three were MOTT positive. Majority of the females in our study were age group of 20–40 years.

8. Discussion

Genital tuberculosis is usually secondary to pulmonary tuberculosis with different modes of transmission: hematogenous (mainly), direct spread from neighboring viscera (gastrointestinal tract, mesenteric lymph node or peritoneum) or direct inoculation of tubercle bacilli during sexual activity if spouse is suffering from tuberculosis of genitalia.[6,8]

Diagnosis of genital tuberculosis is usually elusive. Clinical presentation, abdominal and vaginal examinations do not provide much supportive evidences. Along with these, Mantoux test, Interferon gamma release assay, chest X-ray and erythrocyte sedimentation rate are non-specific and inconclusive. Diverse results on histopathology, laproscopy, hysteroscopy, hysterosalpingography and pelvic ultrasound may create a diagnostic dilemma. Endoscopic procedures are associated with operational risk and can be advised in selected patient with infertility.[6,15]

AFB smear microscopy has poor sensitivity in paucibacillary samples and fail to exclude mycobacterial infection in same. Mycobacterial culture although referred as gold standard, but it neither reduce the TAT to less than 12 days nor improve the positivity in extra pulmonary paucibacillary samples.[16] NAAT has several advantages over older techniques because of good sensitivity and rapidity. In alliance with other modalities, it helps in detection of *Mycobacterium tuberculosis* in endometrial samples[17] However, this technique is not easily accessible in developing country like India due to costlier equipment and infrastructure. Inability to differentiate between live and dead bacilli, is another drawback of this technique.[18]

In our study, only one sample was AFB smear positive (overall 0.22%, 5.5% in 18 positive samples) reflecting a sensitivity of 14.49%. Other studies also corroborate the finding of such low positivity ranging from 0.1 to 5.6%.[19–25] Low sensitivity of AFB smear may be due to the paucibacillary nature of the sample that leads to sparse n uneven distribution of AFB.[23]

Present study showed overall culture positivity of 1.59%. Several studies conducted nationally and internationally have reported positivity ranging from 3.2% to 15.18%[20–24] This can be explained with the presence of less number of AFB in these samples as well as non-representative nature of the sample as only 50–60% cases, the endometrium is affected as compared to fallopian tube (95–100%) and cyclical shedding of endometrium prevents complete development of granuloma in young females.[8] Tubal biopsy is more reliable sample, but difficult to get than endometrium as far as yield of positive culture is concerned.

Culture positivity was less in our study in comparison to other studies. The reason could be 1) These studies were conducted in hospital set up and stringency on selection of study group of patients was maintained 2) Our study was done in lab receiving samples from all gynecological centers including hospitals and Infertility centers 3) Complete clinical history and diagnostic work up of patients were not available therefore patients were categorized based on history of infertility only.

Overall 3.6% samples were found positive for TB PCR (16 out of 438) and among 18 positive samples, TB PCR accounted for 88.8% positivity. Our values are at variance with other studies wherein the values have ranged between 22.2% –85.44%.[19,21,22,24,25] In these studies, all possible diagnostic modalities were taken including TB-PCR with complete clinical history and findings of laproscopy, hysteroscopy and HSG. Sensitivity is very high in these cases with strong clinical suspicion. In our study, complete clinical history of the patients was not available, therefore all cases with history of infertility were studied for genital tuberculosis. Other causes of infertility in all such patients cannot be ruled out.

Five samples were found MTB culture and TB-PCR positive and two NTM culture positive samples were TB-PCR negative. The reason for TB-PCR negative could be presence of PCR inhibitors, blood or portion of the sample tested for TB PCR might not have bacilli, but clinical significance of NTM in a single endometrial sample is also questionable due to ubiquitous presence of these organisms and treatment guidelines are still not available.

Table 2 – Overall positivity along with positivity in positive samples by various methods.

Sample tested- 438	ZN smear microscopy	Culture	TB PCR	ZN smear microscopy + culture positive	Culture + TB PCR positive	Culture negative TB PCR positive	ZN smear, culture and TB PCR positive
Number of Samples positive	1	7	16	1	5	11	1
Percentage positivity of all positive samples (Total positive- 18)	5.5%	38.8%	88.8%	5.5%	22.2%	61.1%	5.5%
Overall positivity	0.22%	1.59%	3.6%	0.22%	1.14%	2.5%	0.22%

The reason of low overall positivity in endometrial samples could be due to non-representative sample, focal lesion or if the sample was not collected in late secretory phase of menstrual cycle usually it should be on 24–26th day of menstrual cycle day or first 12 hours of onset of menses as granuloma takes 2 weeks to develop.[8,19,27] In our study, most of the samples were missing with the menstrual history and last menstrual period, therefore if appropriate time of collection is strictly followed, the positivity rate may be increased. This needs to be studied further.

Even though overall positivity by TB PCR is less, but sensitivity and specificity of TB PCR was found 71.43% and 97.54% respectively that was in line with other studies also.[23,26]

Studies	TB-PCR Sensitivity
S Sethi et al (2016)[23]	95.59%
Gunjan Shrivastava et al (2014)[26]	82%

Eleven samples were TB PCR positive, culture negative, this could be due to less bacterial load or dead bacilli that could be detected by TB PCR but not in culture. False positive TB PCR should be read cautiously as there is no gold standard test other than culture for diagnosing genital tuberculosis and comparing with TB PCR.[28]

As we found in our study that sensitivity of both ZN microscopy and culture is very low, TB PCR was good in sensitivity, but overall positivity of samples was very less. TB-PCR can be promising by submitting right sample collected from right site and right time of menstrual cycle and its sensitivity can further be improved. On other side, it was noted that we cannot completely rely on a single molecular modality for the diagnosis of genital tuberculosis. Only those patients with high clinical suspicion of genital tuberculosis should be evaluated and treated specially ruling out other possible causes of tubal infertility like *C. trachomatis* and *N. gonorrhea*.[28] One must go for all diagnostic possibilities with minimum patient discomfort to rule in and out tuberculosis in patient with infertility.

9. Limitations

There were few limitations of the study: First, the study was performed retrospectively, therefore.cases couldn't be correlated with the clinical status of the patient other than infertility as.mentioned in the clinical history. Second, all required data was gathered from TRF and most of.the cases were missing with radiological, laparoscopic, hysteroscopic findings and histo-pathological reports. Because of this, these parameters were not included and compared.

10. Conclusion

Genital tuberculosis is still rampant in India and there are many challenges associated with the diagnosis of this disease. In our study also, it is presented with the same picture. Low positivity rate of endometrial samples by using different modalities still put a question mark on the possibility and the actual presence of the disease. Tuberculosis is endemic in India and one cannot deny on the large prevalence of latent tuberculosis in community, but before treating any female for genital tuberculosis, it should be confirmed clinically and by diagnostic methods. For this each modality will act as an adjunct to other. Appropriate sample type also plays a crucial role. Therefore, one should include all possible modalities to reach the final diagnosis and try to allay the anxiety of a female with infertility and unnecessary exposure of side effects and drug toxicity of ATT.

Conflicts of interest

The authors have none to declare.

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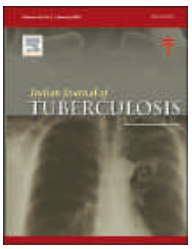

Original Article

Factors affecting outcomes of individualised treatment for drug resistant tuberculosis in an endemic region

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ABSTRACT

Background: Individualised treatment regimens for drug resistant tuberculosis have improved outcomes. This retrospective observational study examined potential factors that affect individualised treatment in an endemic region, and highlighted predictors of a successful outcome.

Methods: We examined records of proven MDR, pre-XDR and XDR TB patients diagnosed and started on treatment between 2010 and 2014, and collected the following data for each patient: age, gender, comorbidities, past history of TB, diagnosis, site of disease, drug susceptibility testing (DST) results, treatment, adverse reactions to anti-tubercular drugs, treatment changes and outcomes, which were recorded as positive, negative or neutral. Tests of association were carried out between factors and outcomes, following which multiple logistic regression analysis was done to determine the predictors of a positive outcome such as patient cured after completion of treatment at 18 months or longer.

Results: Fifty-nine patients completed treatment at our centre. The median age was 26 years (range 8–65 years). There were 31 (52.5%) female patients. Forty-four (74.6%) were successfully treated over a median treatment period of 23 months (range 18–30 months). Successful outcomes were associated with age less than 45 years ($P = 0.01$, OR = 6.67, 95% CI = 1.73–23.47), resistance to fewer than five drugs ($P = 0.001$, OR = 9.51, 95% CI = 2.50–38.18) and susceptibility to Group 4 drugs ($P = 0.04$, OR = 4.71, 95% CI = 1.03–16.83).

Conclusions: Age and drug susceptibility were important predictors of treatment outcome.

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1. Introduction

India has a high burden of multidrug resistant (MDR), pre-extensively drug resistant (pre-XDR), and extensively drug resistant (XDR) tuberculosis (TB), with nearly 71,000 notified pulmonary MDR TB cases alone in 2014, of which 25,748 were new laboratory confirmed cases.[1] These numbers represent a threat to national tuberculosis control efforts, besides being a potential source for global spread of the disease. Challenges to treating drug resistant tuberculosis stem from long treatment durations and include default and loss to follow up, adverse reactions to anti-tubercular drugs and treatment failure, all leading to a vicious cycle with amplifying drug resistance at its core.[2]

Individualising tuberculosis treatment to unique patient and disease situations improves outcomes in both resource-rich non-endemic settings, and in populous resource-poor endemic regions with varied and unpredictable patterns of drug resistance.[2–7] Guidelines recommend the initiation of empirical treatment based on clinical and epidemiological characteristics, treatment history and past drug susceptibility testing (DST) results, followed by individualisation of treatment regimens once the latest DST results become available.[3,8,9] Studies show a consistent association between in vitro susceptibility of individual drugs, their use in therapy and consequent treatment success.[4,5,10–12] DST is therefore central to individualising tuberculosis treatment. However, DST is performed for only 12% of new bacteriologically confirmed cases and 58% of retreatment cases globally.[1] In India, only 25% of MDR TB cases have DST results to fluoroquinolones and an injectable agent.[1] Studies also show that outcomes of treatment regimens, both individualised as well as standardised, may be influenced by a host of demographic, epidemiological and clinical factors that vary between settings.[2,4,5]

In view of increasing drug resistance and lack of standardised DST in most resource-poor endemic regions, as well as the reported success rates of individualised treatment regimens for drug resistant tuberculosis, there is a clear need to evaluate the factors that affect individualised treatment, and a need to determine the best predictors of treatment outcomes in such settings. There is a lack of literature on this subject from India. Hence, this is what we aimed to establish in this retrospective observational study.

2. Methods

2.1. Study subjects

This study included patients who were diagnosed and started on treatment for MDR, pre-XDR and XDR TB at our centre between 2010 and 2014, and for whom DST had been performed. Patients who started treatment before January 2010 and those who had not completed treatment by July 2016 were excluded.

The Institutional Ethics Committee at our centre approved this study.

Identification of *M. tuberculosis* in our study cohort was carried out by a combination of Cepheid TB GeneXpert® System and BD BACTEC™ MGIT™ 960 System or cultures on Löwenstein-Jensen medium. Preliminary DST results were obtained by genotypic resistance profiling (Cepheid TB GeneXpert® System or Hain line probe assays: Lifescience GenoType MTBDR*plus* and GenoType MTBDR*sl* Systems). Final DST was carried out by phenotypic methods (in vitro DST on Löwenstein-Jensen medium by the resistance ratio method in cases from 2010 to early 2012, and using the BD BACTEC™ MGIT™ 960 System in cases from 2012 onwards).

2.2. Definitions

MDR TB was defined as tuberculosis resistant to at least isoniazid and rifampicin; pre-XDR TB as resistant to isoniazid, rifampicin and either a fluoroquinolone or second line injectable agent but not both; and XDR TB as resistant to isoniazid, rifampicin, any fluoroquinolone and at least one of three injectable second line drugs, kanamycin, amikacin or capreomycin.

Anti-tubercular drugs were categorised into the five World Health Organisation (WHO) groups.[8] The new categorisation introduced by WHO in mid-2016[13] was not used as patients enrolled in this study had completed treatment before then, and had been treated according to the previous guidelines.

A treatment change included any unexpected and unplanned addition, subtraction or substitution of a drug in a patient's treatment regimen.

Only those adverse reactions to anti-tubercular drugs were considered that were severe enough to warrant alteration of dosage regimens or withdrawal of the implicated drugs, i.e., grade 3 to grade 4 treatment-associated adverse events (Common Terminology Criteria for Adverse Events Version 4.0, http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf).

2.3. Treatment regimens

In accordance with WHO guidelines, empiric second line tuberculosis treatment (including ethambutol, pyrazinamide, kanamycin, levofloxacin, cycloserine and ethionamide) was initiated for all patients with a clinical suspicion of MDR TB, or showing rifampicin resistance on TB GeneXpert® or line probe assay. This was continued till DST reports were available, following which treatment regimens were individualised.[8,9] Treatment regimens comprised a combination of at least five effective drugs for an intensive phase of at least eight months.[9,14] For patients who could not be administered at least five effective first and second line drugs or those with extensive disease, third line drugs (clofazimine, clarithromycin, linezolid or amoxicillin-clavulanate) were included in the regimens.[8,9] The patients received daily doses of all medications. Before initiating treatment, all patients were educated about the prognosis and the importance of compliance with treatment. Treatment was given for 18–30 months and patients were followed up at regular intervals on an outpatient basis.

Surgical resection was advised for localised pulmonary lesions. Medical treatment was continued post resection.

2.4. Data collection

Patient records were examined and the following data collected for each patient: age, gender, comorbidities, past history of TB, diagnosis, site of disease, DST results, treatment, adverse reactions to anti-tubercular drugs, treatment changes and outcomes.

2.5. Outcomes

After stopping treatment, patients were followed up at least twice for the first six months and six monthly thereafter.

Treatment outcomes were adapted from RNTCP and WHO guidelines, as well as standard outcomes collected for MDR TB surveillance in the UK[3,8,15]:

1. *Cure*: Completing a full course of treatment by 18 months or longer resulting in clinical cure (resolution of symptoms), microbiological resolution (smear or culture conversion in sputum positive patients), or radiological resolution (documented clearing of disease in X-ray and/or CT scan, in comparison with radiological findings at the beginning of treatment).
2. *Treatment failure*: No change in or worsening of patient's clinical condition, or a final culture is positive, or poor radiological response, after at least 24 months of anti-tubercular treatment.
3. *Death*: Caused by, or contributed to by TB, or relationship between the two unknown.
4. *Default*: Treatment found to have been stopped for at least two consecutive months without justifiable reason or patient lost to follow up.
5. *Transfer out*: Responsibility for patient care was transferred to RNTCP DOTS Plus or back to the referring care provider.

Outcomes were categorised as follows[3,16]:

1. *Positive*: patient cured after completion of treatment at 18 months or longer;
2. *Negative*: treatment failure, default, or death;
3. *Neutral*: patient transferred out.

2.6. Statistical analysis

Results were expressed as number (percentage) or, for data that did not follow a normal distribution, median and interquartile range (IQR). Categorical variables were analysed by Fisher's Exact Test and continuous variables by the Mann–Whitney Test. Patients with neutral outcomes were excluded from this part of the analysis. Variables that had even a possible weak independent association with outcomes ($P < 0.1$) were selected for a multiple logistic regression model to determine the predictors of a positive outcome.

All statistical analyses, except for the multiple regression analysis, were performed on GraphPad Prism version 7 (La Jolla, USA). Multiple logistic regression analysis was performed on Stata/SE 14 (College Station, USA).

3. Results

This study comprised a total of 77 patients who presented to our centre during the study period, of which 59 (76.6%) patients received anti-tubercular treatment at our centre for the entire duration of their illness, and 18 (23.4%) were transferred out. Forty-four of these 59 patients (74.6%) were successfully treated over a median treatment period of 23 months (range 18–30 months). Their outcomes are presented in Table 1. Percentages have been calculated separately for patients treated at our centre and for those transferred out.

The median age of the 59 patients treated at our centre was 26 years (range 8–65 years). There were 31 (52.5%) female patients. Their baseline characteristics are summarised in Table 2. None of the patients were infected with the human immunodeficiency virus (HIV).

By definition all *M. tuberculosis* isolates in the study were resistant to rifampicin and isoniazid. Table 3 summarises their drug resistance patterns. The row totals are different from the total number of patients as not all isolates had DST results to all anti-tubercular drugs. Isolates were resistant to a median of three drugs (range 0–9) besides isoniazid and rifampicin. There were two cases of XDR TB (3.4%) and 18 cases of pre-XDR TB (30.5%).

Sixteen of the 59 patients (27.1%) experienced grade 3 to grade 4 adverse drug reactions to anti-tubercular drugs. Six patients (10.2%) had deranged liver function and one (1.7%) had arthralgia caused by pyrazinamide. Three patients (5.1%) had tendinopathies caused by fluoroquinolones. One patient had acute renal failure, one sensorineural hearing loss and another had a hypersensitivity reaction caused by aminoglycosides (1.7% each). The bacteriostatic drugs caused adverse reactions in four patients (6.8%) with one suffering from psychiatric depression caused by cycloserine, one reporting ethionamide associated photophobia, one para-aminosalicylic acid (PAS) associated leukopenia and another reporting hypothyroidism (it was unclear if PAS or ethionamide was responsible). The Group 5 drugs also caused adverse drug reactions with two patients reporting linezolid associated peripheral neuropathies (3.4%), one linezolid associated hypersensitivity and one clofazimine associated xeroderma (1.7% each).

Table 1 – Outcomes of treatment.

Positive outcome, N = 59 (%)		Negative outcome, N = 59 (%)		Neutral outcome, N = 18 (%)	
Cured after treatment for 18 months or longer	44 (74.6%)	Treatment failure	8 (13.6%)	Transferred to DOTS Plus	16 (88.9%)
		Default	1 (1.6%)	Transferred to referring care provider	2 (11.1%)
		Death	6 (10.2%)		

Table 2 – Baseline characteristics.

Characteristic	N = 59 (%)
Age in years: median, range	26, 8–65
0–15	5 (8.5%)
16–44	44 (74.5%)
45–64	9 (15.3%)
≥65	1 (1.7%)
Gender	
Female	31 (52.5%)
Patients with comorbidities	
Yes[a]	9 (15.3%)
Type 1 Diabetes Mellitus	1
Type 2 Diabetes Mellitus	6
Hypertension	2
COPD[b]	1
Hypothyroidism	1
Interstitial lung disease	1
Rheumatoid arthritis	1
Past history of tuberculosis	20 (33.9%)
Site of disease	
Pulmonary	51 (86.4%)
Extrapulmonary	8 (13.6%)

[a] Two patients had multiple comorbidities.
[b] Chronic Obstructive Pulmonary Disease.

Table 3 – Drug susceptibility patterns.

Drug (N = total tested)	Resistant (%)[a]	Sensitive
Ethambutol (N = 57)	36 (63.2%)	21 (36.8%)
Pyrazinamide (N = 45)	38 (84.4%)	7 (15.6%)
Streptomycin (N = 51)	43 (84.3%)	8 (15.7%)
Kanamycin (N = 57)	3 (5.3%)	54 (94.7%)
Capreomycin (N = 50)	2 (4%)	48 (96%)
Amikacin (N = 36)	4 (11.1%)	32 (88.9%)
Ethionamide (N = 50)	19 (38%)	31 (62%)
Cycloserine (N = 14)	0	14 (100%)
Para-aminosalicylic acid (N = 51)	16 (31.4%)	35 (68.6%)
Moxifloxacin (N = 31)	17 (54.8%)	14 (45.2%)
Ofloxacin (N = 45)	22 (48.9%)	23 (51.1%)
Levofloxacin (N = 14)	7 (50%)	7 (50%)
Clofazimine (N = 27)	0	27 (100%)
Clarithromycin (N = 3)	0	3 (100%)

[a] The figures in parentheses indicate the percentage of tested isolates. Not all drugs were tested for each isolate.

Fourteen (23.7%) patients had changes to their treatment regimens at some point during treatment, all in response to side effects or drug intolerance. A median of one change was made (range 1–4) in these fourteen patients.

Successful outcomes were associated with age less than 45 years ($P = 0.01$, OR = 6.67, 95% CI = 1.73–23.47), resistance to fewer than five drugs ($P = 0.001$, OR = 9.51, 95% CI = 2.50–38.18) and susceptibility to Group 4 drugs ($P = 0.04$, OR = 4.71, 95% CI = 1.03–16.83). The effects of demographic and clinical characteristics on treatment outcomes are presented in Table 4, and the effects of drug resistance patterns and treatment management in Table 5. Results of the multiple logistic regression analysis are presented in Table 6.

4. Discussion

Eighteen of the 77 patients were transferred out, 16 (20.8%) to RNTCP DOTS Plus as they were unable to afford individualised treatment, and two back to their referring care providers. The inability of a considerable proportion of patients to afford individualised treatment represents a potential pitfall in the delivery of individualised treatment regimens in resource-poor endemic regions.[17]

Of the remaining 59 patients who were directly under our care for the duration of their illness, 74.6% were cured of the disease after receiving treatment for 18 months or longer (Table 1). This is very close to the WHO target success rate of ≥75%.[1] In comparison, a recent study from Mumbai reported a success rate of 68% and a study from New Delhi a success rate of 48%,[5,17] whilst a few studies from resource-rich non-endemic regions reported success rates ranging from 66% to 70.6%.[3,4,18] The mortality rate in our study was 10.2%, with death being the outcome in six patients (Table 1). Although this is higher than the mortality rates of 6%-7% reported from non-endemic regions, it is still much less than the overall mortality rate of 21% from MDR TB reported by WHO in the Southeast Asia region in 2012.[1,3,4,18]

Our patients belonged to the age range 8–65 years, with a median age of 26 years and a majority (74.5%) in the 16–44 years age group (Table 2). A Mann–Whitney test returned a P value of 0.14, indicating only a probable weak association

Table 4 – Effect of demographic and clinical characteristics on treatment outcomes.

	Positive treatment outcome (%)	Negative treatment outcome (%)	Odds Ratio (95% CI)	P value
Age			6.67 (1.73–23.47)	0.01
Younger than 45 years (N = 49)	40 (81.6%)	9 (18.4%)		
Older than 45 years (N = 10)	4 (40%)	6 (60%)		
Gender			0.35 (0.11–1.25)	0.1
Female (N = 31)	26 (83.9%)	5 (16.1%)		
Male (N = 28)	18 (64.3%)	10 (35.7%)		
Comorbidities			0.35 (0.08–1.33)	0.21
With comorbidities (N = 9)	5 (55.6%)	4 (44.4%)		
Without comorbidities (N = 50)	39 (78%)	11 (22%)		
Past history of TB			0.7 (0.23–2.46)	0.75
Present (N = 20)	14 (70%)	6 (30%)		
Absent (N = 39)	30 (76.9%)	9 (23.1%)		
Site of disease			0.38 (0.03–2.61)	0.67
Pulmonary (N = 51)	37 (72.5%)	14 (27.5%)		
Extrapulmonary (N = 8)	7 (87.5%)	1 (12.5%)		

Table 5 – Effect of drug resistance patterns and treatment management on treatment outcomes.

	Positive treatment outcome (%)	Negative treatment outcome (%)	Odds Ratio (95% CI)	P value
Drug resistance[a]				
Resistance to <5 drugs (N = 42)	37 (88.1%)	5 (11.9%)	9.51 (2.50–38.18)	0.001
Resistance to Group 1 drugs[b]	36 (73.5%)	13 (26.5%)	(0.29–infinity)	0.56
Sensitive to Group 1 drugs[b]	4 (100%)	0		
Resistance to Group 2 drugs	0	1 (100%)	(0.14–infinity)	0.44
Sensitive to Group 2 drugs	19 (57.6%)	14 (42.4%)		
Resistance to Group 3 drugs	7 (41.2%)	10 (58.8%)	3.93 (0.80–14.33)	0.09
Sensitive to Group 3 drugs	11 (73.3%)	4 (26.7%)		
Resistance to Group 4 drugs	7 (38.9%)	11 (61.1%)	4.71 (1.03–16.83)	0.04
Sensitive to Group 4 drugs	12 (75%)	4 (25%)		
Treatment regimens with				
Group 1 drugs (N = 42)	34 (81%)	8 (19%)	3 (0.85–10.19)	0.1
Group 2 drugs (N = 57)	42 (73.7%)	15 (26.3%)	0 (0–6.4)	>1
Group 3 drugs (N = 54)	40 (74.1%)	14 (25.9%)	0.71 (0.05–5.1)	>1
Group 4 drugs (N = 57)	43 (75.4%)	14 (24.6%)	3.07 (0.15 – 59.45)	0.45
Group 5 drugs (N = 42)	28 (66.7%)	14 (33.3%)	0.13 (0.01–0.86)	0.04
Surgery[c]	3 (100%)	0		
Adverse drug reactions (N = 17)	13 (76.5%)	4 (23.5%)	1.15 (0.34–3.77)	>1
Treatment changes (N = 15)	11 (73.3%)	4 (26.7%)	0.92 (0.26–3.05)	>1

[a] Not all isolates were tested against all drugs. Sensitivities were calculated for each group if DST reported on at least one drug of the group.
[b] Excludes isoniazid and rifampicin.
[c] Three patients were advised surgical treatment (lobectomy) and tuberculosis treatment. All were cured.

Table 6 – Multiple logistic regression analysis of factors affecting a successful outcome.*

Covariable	P value	Odds ratio (95% CI)
Age less than 45	0.02	15.31 (1.69–138.99)
Female gender	0.37	2.09 (0.42–10.36)
Resistance to less than 5 drugs	0.01	12.43 (2.04–75.88)
Treatment with Group 1 Drug	0.25	2.51 (0.52–12.17)
Treatment with Group 5 Drug	0.25	0.2 (0.01–3.19)

* Not all patients had DSTs to each of the four groups, therefore susceptibilities to Groups 3 and 4 could not be included in this model although univariate analysis returned $P < 0.1$ as seen in Table 5.

between age and treatment outcomes. However, on categorising our data in the same manner as Anderson et al.,[3] we found that patients younger than 45 years were significantly more likely to succeed with individualised treatment (Table 4). This result matched the results obtained by Anderson et al. in their nationwide cohort study in the UK, which showed that patients in the 15–44 years age group were most likely to succeed.[3] However, as we received only one geriatric patient, aged 65 years, and five paediatric patients (younger than 15), this study was unable to provide a more accurate measure of outcomes in these age groups.

Similar to previous studies, we obtained higher cure rates in female patients, but this was not statistically significant (Table 4).[3,4]

Only 15.3% of patients had comorbid conditions with type 2 diabetes mellitus being the commonest, and only 33.9% of patients had a history of tuberculosis in the past (Table 2). Unlike a number of previous reports, the presence of comorbid conditions and a past history of tuberculosis did not significantly impact outcomes in our study (Table 4).[3,7,19] This could be attributed to the fact that we had few patients with comorbidities and a past history of tuberculosis, and the relatively young age group to which most of our patients belonged.

We had a total of eight patients with drug resistant extra-pulmonary tuberculosis: four with cervical lymphadenitis, two with tuberculous peritonitis, one with axillary lymphadenitis and one with intestinal tuberculosis. Similar to the UK cohort study, patients with extra-pulmonary tuberculosis appeared to have a higher cure rate than those with pulmonary disease,[3] but a statistically significant association was not found due to the small number of patients with extra-pulmonary disease in our study (Table 4).

We noticed considerable variation in drug susceptibility testing and not all patients had DST results to all drugs as is evident from Table 3. Of the isolates tested against these drugs, most were resistant to the Group 1 drugs ethambutol and pyrazinamide, to streptomycin, and to the Group 3 drugs (fluoroquinolones). Maximum isolates were sensitive to the second line aminoglycosides and, although considerable, resistance to Group 4 drugs was not very high. Our results are similar to trends observed in a recent large-scale study in the Mumbai region.[20] However, resistance patterns are different in non-endemic regions, with generally greater resistance to aminoglycosides and lesser resistance to ethambutol and fluoroquinolones. The resistance rate to the Group 4 bacteriostatic drugs obtained in our study is similar to rates in studies from both endemic and non-endemic regions.[3,4,10,20] In contrast to local trends, we encountered relatively few patients with pre-XDR (30.5%) and XDR TB (3.4%).[20]

As in previous studies, resistance to fewer than five drugs was significantly associated with a successful treatment outcome.[3,4,17]

Uniquely, we found that there was a significant association between in vitro susceptibility to Group 4 drugs and treatment success (Table 5). This could be because of the high prevalence of resistance to Group 1 drugs and fluoroquinolones in our

study, making the Group 4 drugs important arbiters of treatment outcomes.

However, we did not find a statistically significant association between treatment with Group 4 drugs and positive outcomes. Drawing a comparison was difficult because these drugs, being an essential component of MDR TB therapy,[9] were taken by nearly all patients in both outcome groups. Whilst we recognise that there is a difference between in vitro susceptibility and in vivo activity of these drugs, our results nevertheless demonstrate a need to perform DST for these drugs in the setting of high level resistance to Group 1 and Group 3 drugs.[10,21,22]

Treatment with Group 1 drugs resulted in higher cure rates, and although this was not statistically significant, it reemphasises the importance of Group 1 drugs in tuberculosis treatment (Table 5).

Group 5 drugs were taken by a total of 42 patients. Treatment requiring Group 5 drugs resulted in significantly lower cure rates. We believe this could be related to patient and disease factors: 13 patients had isolates that were resistant to five or more drugs, 11 required treatment changes due to drug intolerance and the remaining 18 had either extensive disease or responded poorly to other anti-tubercular drugs. Few studies show the efficacy of Group 5 drugs and further evaluation of their potential is required, especially in the present scenario of rising pre-XDR and XDR TB.[23]

All patients had some minor adverse reactions to anti-tubercular drugs, particularly acidity and skin hyperpigmentation. Only 27.1% experienced adverse drug reactions severe enough to warrant changes in treatment (Table 5). However, neither adverse drug reactions nor treatment changes had a significant impact on treatment outcomes.

Multiple logistic regression analysis showed that age less than 45 years and resistance to less than five drugs were the best predictors of a successful treatment outcome in our setting (Table 6). We were unable to include susceptibility to Group 4 drugs in this model as DST results were not available for all patients.

Our study has a few important drawbacks. DST is not standardised across laboratories in India. This accounted for the heterogeneity in drug susceptibility patterns in our study cohort. Our cohort included very few paediatric and only one geriatric patient, making it difficult to measure outcomes in these vulnerable age groups. As ours is not a DOTS centre and patients are followed up at intervals, we could not directly observe daily administration of drugs. It is also possible that adverse drug reactions may have been underestimated. Lastly, we could not examine the influence of socio-economic factors as details were not available for many of our patients. Further studies that examine these are required.

In conclusion, we observed a good success rate with individualised treatment in our setting, with patient age and drug susceptibility being important predictors of outcome. We also felt the need for standardisation of DST across laboratories and the inclusion of Group 4 drugs in testing.

Conflicts of interest

The authors have none to declare.

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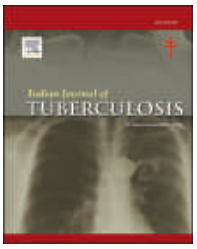

Original article

Isoniazid mono-resistant tuberculosis: Time to take it seriously

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ABSTRACT

Background/Aims: In drug resistant tuberculosis (DRTB) suspects, rifampicin resistance has always been prioritized, hence Cartridge Based Nucleic Acid Amplification Test (CBNAAT) is recommended under Revised National Tuberculosis Control Programme (RNTCP), India. However, since it doesn't detect isoniazid resistance, rifampicin sensitive patients with unknown isoniazid status may be erroneously treated as drug sensitive TB, leading to poor treatment outcomes and emergence of multidrug resistant (MDR) TB. Hence isoniazid mono-resistance should be specifically looked for and treated as per recommendations.

The objective of the present study, almost the first of its kind in India, was to evaluate the burden of isoniazid mono-resistance amongst patients diagnosed with DRTB and to study the association of different patient and disease related factors with treatment outcomes under the treatment regimen specific for isoniazid mono-resistance, started from January 1, 2017 in our state, under RNTCP.

Methods: It was a retrospective study which scrutinized medical records of 52 isoniazid mono-resistant TB patients started on treatment under RNTCP between January 1 to December 31, 2017. Necessary information on possible patient and disease related predicting factors like gender, age, type of mutation (katG/inhA), weight band (26–45 kg/46-70 kg), total serum protein/albumin levels, previous history of anti-tubercular treatment (ATT), history of smoking, HIV status, presence of diabetes mellitus (DM), presence of anemia, occurrence of adverse drug reactions (ADR) during treatment and duration of intensive phase (IP), was retrieved. These factors were analyzed for their possible association with treatment outcomes.

Results: Out of 103 DRTB patients enrolled, 50.5% (52/103) patients were diagnosed with isoniazid mono-resistance. 50/103 were MDR-TB and 1/103 were extensively-drug resistant TB (XDR-TB). Further analysis of these 52 isoniazid mono-resistant patients revealed:35 (67.3%) were males and 17 (32.7%) females. 27 (51.9%) patients were <30 years, 25 (48.1%) being ≥30 years of age. All patients were negative for HIV. 34/52 (65.4%) patients were declared cured, 15/52 were lost to follow up (LTFU) and 3/52 died (1 male, 2 females). Excluding these 3 patients who died, cure rates were significantly better in females (14/15 = 93.3%), with only 1/15 LTFU, than males (20/34 = 58.8% cure, 14/34 = 41.2% LTFU),

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(p = 0.019). Patients who were <30 years of age had significantly better cure rates (21/25 = 84%) with lesser LTFU's (4/25 = 16%), than those ≥30years of age (13/24 = 54.2% cure, 11/24 = 45.8% LTFU), (p = 0.032). Review of previous history of ATT revealed that 33 patients had primary isoniazid mono-resistance, 4 patients had previous history of being LTFU, 9 had recurrent TB and 3 were labeled as failure. Cure rates were significantly better in primary isoniazid mono-resistant patients (26/33 = 78.8%), than those with previous history of being LTFU(0/4), (p = 0.04).

Type of mutation, weight band, total serum protein/albumin, history of smoking, presence of DM, presence of anemia, occurrence of ADR and duration of IP did not affect treatment outcomes.

Conclusion: Isoniazid mono-resistance formed a major chunk of DRTB, with majority of the patients detected with primary mono-resistance. Strategically framed treatment regimens for isoniazid mono-resistance under RNTCP in India are effective in a wide range of population. Still, there are high chances of LTFU/default, which needs to be addressed on priority. Male gender, age ≥30 years and being LTFU in the past are associated with poorer cure rates, hence should be paid special attention.

1. Introduction

Tuberculosis remains a global public health problem, as it is a major cause of morbidity and mortality. India constitutes one of the high burden countries for Tuberculosis. Emergence of drug resistant TB has posed newer challenges for the TB control.[1,2] Estimation of the pattern of DRTB, and data on the efficacy of therapy, by evaluation of treatment outcomes is important. Information so generated, from different local areas will help in the formulation of effective and reliable interventional policies specifically for our country.[3]

Isoniazid, because of its potent bactericidal activity, is an important first line drug in the management of TB. Isoniazid resistance singly or in combination with other drugs, is a common type of resistance worldwide, but has not been prioritized much.[4–7] The adequacy of standard first line treatment for TB in such cases, comes under question, and needs to be thoroughly studied.[8] There is ample data which suggests that isoniazid mono-resistance, if unidentified/mistreated, may increase the chances of progression to MDR TB and poorer treatment outcomes.[4,7,9–14] Hence there is an urgent need to study the isoniazid mono-resistant TB population in detail, right from diagnosis and management, to the final treatment outcomes, and thus provide data for future planning of TB control strategies at the national level.[14]

The nodal DRTB centre in our state, is one of the centers where regimen for isoniazid mono-resistance was launched on January 1, 2017, under Revised National Tuberculosis Control Programme (RNTCP), India. Isoniazid mono-resistance is specifically looked for in patients whose sputum/bronchoalveolar lavage (BAL) is found to be rifampicin sensitive on CBNAAT, by subjecting the sample for Line probe assay (LPA) and is treated accordingly. The present study was hence done to estimate the burden of isoniazid mono-resistance amongst patients with DRTB and evaluate the association of different patient and disease related factors with treatment outcomes.

2. Materials and methods

It was a retrospective study which scrutinized the medical records of 52 isoniazid mono-resistant TB patients started on treatment regimen specific for isoniazid mono-resistance under RNTCP between January 1, 2017 to December 31, 2017 at the nodal DRTB centre, Department of Pulmonary Medicine, Government Medical College and Hospital, Chandigarh.

Necessary information on possible predicting factors like age, gender, type of mutation (katG/inhA), weight band (25–45 kg/46-70 kg), hemoglobin levels, total serum protein and albumin levels, previous history of anti-tubercular treatment (ATT), history of smoking, HIV status, presence of diabetes mellitus (DM), occurrence of adverse drug reactions (ADR) during treatment and duration of intensive phase (IP) as determined by sputum conversion (3/4/5/6 months), was retrieved. These factors were analyzed with relation to the treatment outcomes.

Anemia was defined as a Hb < 13 g/dl in males and <12 g/dl in females, hypoproteinemia as total serum proteins <6 g/dl and hypoalbuminemia as total serum albumin<3.5g/dl.[15–17] Diabetes mellitus was defined as fasting plasma glucose (FPG) ≥126 mg/dL or glycated hemoglobin ≥6.5%.[18] In previously treated patients, past history of ATT was defined as per Technical Operational Guidelines (TOG) and categorized into lost to follow up (LTFU), recurrent TB and treatment failure.[19]

Patients were given treatment as per TOG.[19] The total duration of treatment varied from 9 to 12 months. The intensive phase (IP) was for 3 months and extendable up to a maximum of 6 months as per requirements. The continuation phase (CP) was for a fixed duration of 6 months. The treatment regimen consisted of Kanamycin, Levofloxacin, Rifampicin,

Ethambutol and Pyrazinamide in IP and Levofloxacin, Rifampicin, Ethambutol and Pyrazinamide in the CP. In addition, high dose Isoniazid was added to the regimen if LPA showed inhA mutation.

Treatment outcomes were defined as per TOG guidelines and categorized into cured, died and LTFU.[19]

The study was approved by the institute's ethical committee.

Statistical analysis: Discrete categorical data were presented as n (%). Normality of quantitative data were checked by measures of Kolmogorov Smirnov tests of normality. As age was normally distributed it was represented as mean ± SD & range. Proportions were compared for treatment outcome using Chi square or Fisher's exact test whichever applicable. All statistical tests were two-sided and performed at a significance level of $\alpha = 0.05$. Analysis was conducted using IBM SPSS STATISTICS (version 22.0)

3. Results

Burden: Total 103 DRTB patients were diagnosed during the study period, out of which 50.5% (52/103) patients were diagnosed with isoniazid mono-resistance. 50/103 were MDR-TB and 1/103 was XDR-TB.

Treatment outcomes: The treatment outcomes are depicted in Fig. 1. 65.4% patients had successful treatment outcomes and were declared cured. Out of 15 LTFU's, it was seen that 10 patients were LTFU during IP only and 5 during the CP. Out of 15 LTFU's, 13 were finally labeled as LTFU and never traced, 1 was later switched over to MDR regimen and 1 was reregistered as isoniazid mono-resistant TB.

Patient and disease related factors and their association with treatment outcomes: Gender, age distribution, type of mutation (katG/inhA), weight band (26–45 kg/46-70 kg), total serum protein and albumin levels, history of smoking, presence of diabetes mellitus (DM), presence of anemia and occurrence of adverse drug reactions (ADR) during treatment of these 52 patients is shown in Table 1. All patients were negative for HIV. The previous history of ATT (excluding 3 patients who died) is depicted in Table 2. The duration of IP (in patients who were declared as cured) was 3, 4, 5 and 6 months in 7, 13, 8 and 6 patients respectively, and the median duration of treatment in these patients was 10 months.

Table 1 – Showing baseline characteristics of isoniazid mono-resistant patients (n = 52).

Gender	Males	35 (67.3%)
	Females	17 (32.7%)
Age (years)	<30	27 (51.9%)
	>30	25 (48.1%)
Type of Mutation	katG	39 (75%)
	inhA	13 (25%)
Weight Band (kg)	26–45	34 (65.4%)
	46–70	18 (34.6%)
Total serum protein	Hypoproteinemia	3 (5.7%)
	Normal	49 (94.3%)
Total serum albumin	Hypoalbuminemia	22 (42.3%)
	Normal	30 (57.7%)
Smoking history	Yes	15 (28.9%)
	No	37 (71.1%)
Diabetes Mellitus	Yes	7 (13.5%)
	No	45 (86.5%)
Anaemia	Yes	38 (73.1%)
	No	14 (26.9%)
Adverse Drug reactions	Yes	3 (5.7%)
	No	49 (94.3%)

Excluding 3 patients who died (1 male and 2 females), cure rates were significantly better in females with lesser LTFU's, than males ($p = 0.019$). Patients who were <30 years of age had significantly better cure rates with lesser LTFU's, than those ≥30 yrs of age ($p = 0.032$). Reviewing previous history of ATT, it was seen that majority of the patients (n = 33) were diagnosed with primary mono-resistance to the drug isoniazid and did not have history of ATT consumption in the past, 4 patients had previous history of LTFU, 9 had recurrent TB and 3 were labeled as failure. Cure rates were significantly better in primary isoniazid mono-resistant patients (26/33 = 78.8%), than those with previous history of LTFU (0/4) ($p = 0.04$) (Table 2)

However, type of mutation, weight band, total serum protein/albumin, history of smoking, presence of DM, presence of anemia, occurrence of ADR and duration of IP did not affect the treatment outcomes.

4. Discussion

Isoniazid mono-resistance forms a major chunk of DRTB. The first national anti tuberculosis drug resistance survey from India has revealed the extent of isoniazid mono-resistance to

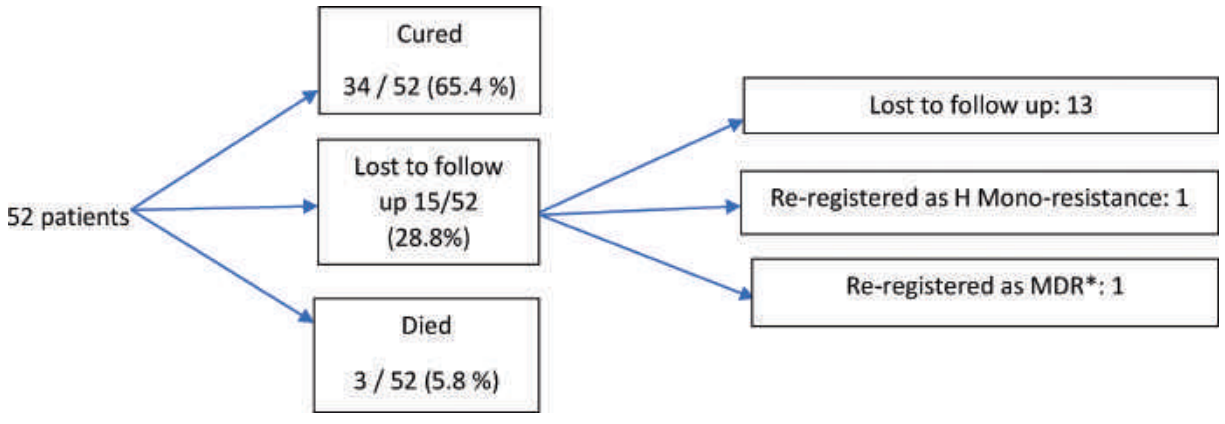

Fig. 1 – Showing the treatment outcomes of isoniazid mono-resistant patients (n = 52).

Table 2 – Showing relationship of treatment outcomes (Cured and Lost to follow up, n = 49) with gender, age and previous history of anti tubercular treatment.

Parameter		Treatment Outcome	
		Cured	Lost to Follow up
Gender	Male	20 (58.8%)	14 (41.2%)
	Female	14 (93.3%)	1 (6.7%)
Age (years)	<30	21 (84%)	4 (16%)
	>30	13 (54.2%)	11 (45.8%)
Previous history of Anti Tubercular Treatment (ATT)	Nil (Primary mono drug resistant) (n = 33)	26 (78.8%)	7 (21.2%)
	Lost to Follow up (n = 4)	0 (0%)	4 (100%)
	Recurrence (n = 9)	6 (66.6%)	3 (33.33%)
	Failure (n = 3)	2 (66.6%)	1 (33.3%)

*: Multi Drug Resistant Tuberculosis.

be 3.85% in new patients, and 7.61% in previously treated patients.[2] The reasons for the emergence of this type of resistance pattern could be many and include inappropriate isoniazid monotherapy in a patient with active TB (misdiagnosed as latent TB infection), previous suboptimal therapy because of non identification of this mono-resistance etc.[20] Though less likely in the Indian context, because of a limited use, the confounding effect of isoniazid preventive therapy on emergence of isoniazid mono-resistance has also been hypothesized.[7,21]

This high a burden of isoniazid mono-resistance out of the total pool of DRTB is alarming, and gives us an indication to consider it seriously and immediately. Had it not been specifically looked for, a substantial portion of the DRTB patients with isoniazid mono-resistance would have been missed. With subsequent mistreatment, there would have been increased chances of poorer treatment outcomes and progression to MDR TB.[4,7,9–14]

Another striking fact revealed from this study is that majority of the patients had primary drug resistance to isoniazid, with no history of exposure to any ATT in the past. For a clinician, and for the program, this highlights the importance of being vigilant, religiously follow the recommendations and actively seek for any hidden isoniazid mono-resistance in new cases. Such patients, if left unidentified or mistreated with standard first line chemotherapy are a threat to the community from the public health view as well, as they will be a continuous source of spread of the resistant tubercular strains in the community.

Majority of the patients in our study were males, the results being in concordance with the predominance of males in the national anti tuberculosis drug resistance survey,[2] and other studies on isoniazid mono-resistance from different parts of the world.[4,14,20] The age distribution of our patients is similar to the existing literature.[2,5,14,20] The median treatment duration, as calculated in patients who were declared as cured was found to be 10 months, and was comparable to other studies.[22]

On analysis of the treatment outcomes, it was seen that majority of the patients had successful treatment outcomes, and were declared cured, indicating that the treatment regimen for isoniazid mono-resistance being provided under the program is highly effective. Treatment outcomes are excellent if patients adhere to the therapy. Other studies have also concluded that carefully tailored and robust treatment regimens for isoniazid mono-resistance are highly successful.[4,6,10,23] However, 15 patients (28.8%) were LTFU, which is an important figure in itself, especially when seen from the point of view of judging the success of the program. The patients who are LTFU serve as a dangerous reservoir of infection for the community. Inadequate treatment in such patients leads to the selection of the drug resistant bacteria and spread of these resistant strains to those in close vicinity.[3]

Another important finding was that all the patients who were LTFU during their previous anti tubercular treatment in the past, again were LTFU during the course of therapy when started on treatment for isoniazid mono-resistance. This suggests that these habitual LTFU's are a major strata of TB patients which needs to be specifically targeted at the individual, community and program level. Curbing widespread dissemination of infection from these patients and treating them efficiently is the need of the hour for an effective TB program in the country. Each patient should be educated and counseled individually, in addition to the community sensitization measures. Possible reasons of LTFU like occurrence of adverse drug reactions, superadded infections, poor nutritional status, lack of family support, social stigma, poor socioeconomic background, rebellious behaviour etc should be specifically looked at. Treatment should be carefully supervised with early addressal of issues if any, and clinicians must ensure completion of treatment.

There were fewer LTFU's, with better cure rates amongst females as compared to males. So, more care needs to be given to ensure better compliance in the male population.

In our study, with respect to age, it was seen that the younger patients (<30 years of age) had better cure rates with lesser LTFU in comparison with their older counterparts. Variable results, both similar, and contradictory, have been observed in other studies.[4,24] Our results indirectly reflect better compliance in the younger age groups. The possible explanations for the same include better family/parental support in taking the medications, more energetic and enthusiastic approach as a result of being young, harboring a positive attitude towards health etc. Patients >30 years of age have an increased tendency of turning to LTFU because of erratic working hours and job profiles, heightened stress due to social problems, migrating out for personal and

professional reasons etc. A variety of factors thus act as barriers to compliance.

All the patients enrolled at our center for treatment were HIV non reactive. Hence the impact of HIV status on treatment outcomes could not be commented upon. Our study found the katG mutations in 75% of patients. In comparison, the national anti tuberculosis drug resistance survey, revealed the katG mutations to the extent of 90%.[2] Though patients with katG mutation are expected to have poorer treatment outcomes in comparison with the patients who have inhA mutations,[5] the treatment outcomes were not affected by the type of mutation in our study. Since the regimen has the provision for different doses as per weight bands, specific management protocols in case of ADR's and extension of the IP as per sputum status, treatment outcomes were unaffected by the weight of the patient, occurrence of ADR's or duration of IP.

As per our analysis, it was seen that total serum protein/albumin, history of smoking, presence of DM and presence of anemia did not affect treatment outcomes.

Our study thus provides important information on the significance of certain factors like gender, age and being LTFU during previous ATT in the past, which can affect the treatment outcomes in patients with isoniazid mono-resistance.

Limitations of the study: The study was retrospective, with analysis of the available records of a single center. It is possible that those patients who continued the treatment somewhere else after leaving it in-between with us (migrated out), were reported as 'LTFU' in our records.

5. Conclusion

Isoniazid mono-resistance is an important and widely prevalent form of DRTB. Primary mono-resistance to isoniazid forms a major proportion of these isoniazid mono-resistant patients. Physicians should be vigilant in promptly identifying and treating isoniazid mono-resistant TB. Adherence to the recommended regimen needs to be specifically addressed on priority as many patients discontinued the treatment and were LTFU. Male gender, age $\geq$30 years and being LTFU in the past are associated with poorer cure rates, hence should be paid special attention for an effective break in the chain of transmission, resultant better treatment outcomes and prevention of emergence of MDR/XDR TB. Treatment for isoniazid mono-resistance, if strictly adhered to, is very effective and is associated with high cure rates.

Conflict of interest

The authors have no conflicts of interest.

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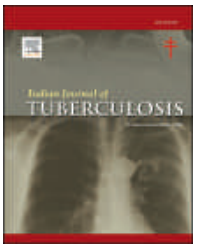

Original article

Strategy to sensitize private practitioners on RNTCP through medico-social workers in urban field practice area of a Medical College in Bengaluru, Karnataka

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ABSTRACT

Background: India accounts for 25% of global TB burden and majority of TB patients seek care from private practitioners. It becomes imperative to involve private practitioners with newer strategies to strengthen the Revised National Tuberculosis Control Program (RNTCP). A study was conducted to assess the knowledge, attitude and practices among private practitioners with regards to tuberculosis case detection and referral and to demonstrate the feasibility of utilizing existing medico-social worker of a medical college in sensitizing the private practitioners.

Methods: An intervention study was conducted during 2017. In an urban field practice area of a medical college, 34 allopathic private practitioners (PP) from six slums formed the study population. The RNTCP trained Medico social workers (MSW) of medical college provided repeated sensitization to private practitioners on case referrals. The data of KAP among private practitioners was collected. The output of repeated sensitization was measured by comparing the number of cases referred by Private Practitioners to DMC during the pre and post intervention period.

Results: Only 1 in 2 practitioners were aware about the duration of cough in presumptive TB cases. Nearly 44% of them were not aware about the first investigation of choice under RNTCP; 53% of the doctors did not know about the total number of sputum samples to be collected. After the sensitization of PPs by MSWs the number of presumptive pulmonary cases was increased by more than two folds.

Conclusion: The strategy of utilizing the services of medico-social workers employed in a medical college to sensitize the private practitioners is feasible and has demonstrated the increase in number of presumptive TB case referrals to DMCs.

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1. Introduction

According the WHO global TB report, the estimated incidence of tuberculosis (TB) in India was about 2.8 million cases accounting for about a quarter of the world's TB cases. In 2015, the private provider notification of tuberculosis cases in India was about 0.19 million; which constituted only 10% of total tuberculosis cases notified. National strategic plan for elimination of TB has set a target of improving the case notification by private sector from 10% to 50% by 2020.[1]

Private practitioners (PP) cater to majority of the patients with tuberculosis. Almost half of patients with tuberculosis in India initially seek help from the private healthcare sector, where diagnosis, treatment and reporting practices often do not meet national or international standards for tuberculosis.[2]

It is estimated that even in the private clinics, about 2–3% of outpatients would be presumptive pulmonary TB cases and they need sputum smear examination for diagnosis of TB.[3] A recent systematic review revealed that in India there is a delay of nearly two months to diagnose a TB case and majority of patients do visit at least three health facilities before diagnosis is made.[4]

Many drug prescription analysis studies have shown that irrational and inappropriate anti-tuberculosis drug regimens are widely prevalent at the private sector.[5] Health care providers inadequate knowledge and not complying to the Revised National Tuberculosis Control Program (RNTCP) guidelines also affects the quality of service.[6] Many published studies have expressed the concern of suboptimal quality of TB care, particularly in the private sector and have strongly recommended for improvement of quality of care as a priority in India.[7] Hence, involvement of private practitioners is an important component to strengthen RNTCP; especially, in identification of presumptive pulmonary TB cases and referring them to Designated Microscopy Centre (DMC) for appropriate investigations. Taking into cognizance of this situation, the RNTCP has even sanctioned Public Private Mix (PPM) coordinators in each district to effectively build and strengthen the linkages between public and private sector. It is imperative for the programme to develop and adopt newer strategies for involvement of private practitioners.

The presence of a medical college each of the districts of Karnataka also provides an ample opportunity for the programme to extend the referral linkages of private practitioners in urban and rural field practice areas of medical colleges. The strategy of utilizing the medical social workers (MSW) from department of community medicine to sensitize the private practitioners and regularly monitor them for appropriate referral of presumptive TB case and TB patients has ample potential to further strengthen the linkages. Based on this strategy, we conducted a study at Vydehi Institute of Medical Sciences and Research Centre, Bengaluru by sensitizing the private practitioners on RNTCP through MSWs. The objectives of the study were to assess the knowledge, attitude and practices about case detection and referral according to RNTCP guidelines among private practitioners in urban field practice area and to assess the effectiveness of MSWs interventions in terms of number of presumptive TB cases referred to Designated Microscopy Centre by Private Practitioners.

2. Methods

2.1. *Settings*

An interventional study was conducted during July through December 2017 at the urban field practice area of Vydehi Institute of Medical Sciences and Research Centre in Bengaluru, which caters to nearly 12,792 population. The urban field practice area has six slums. A total of 36 allopathic doctors from all 6 slums of urban field practice area of Medical College who were practicing in the private clinics were included in this study. The Principal investigator along with one MSW approached the PP's at their clinics to brief them about the study; those who were not available in their clinics even after two visits were excluded from the study. In the area, 34 practitioners (94% response rate) agreed to be part of the study and were enrolled. The institutional ethics committee approval was obtained from Vydehi Institute of Medical Sciences and Research Centre and a written informed consent was obtained from all the participants.

A Medico social worker (MSW) from the medical college was assigned the responsibility of sensitizing and coordinating with the private practitioners. A three day training was provided to the MSW on RNTCP guidelines by Community Medicine faculty and by RNTCP medical officer of medical college on case finding and referral. The gain in knowledge was assessed by administering a questionnaire on the training received.

The Information Education Communication (IEC) material customized to local settings were developed for the training of Medico-Social Worker (MSW) based on RNTCP Guidelines on case finding and referral of presumptive pulmonary TB cases to Designated Microscopy Centre (DMC). A poster and a case referral book were also developed for doctors to refer presumptive pulmonary TB cases. The trained MSW collected data from the private practitioners by using self-administered data collection tool. The tool comprised of questions to elicit details on demographic profile and knowledge about tuberculosis assessment (05 questions), attitudes (06 questions) and practices (08 questions) adopted with respect to RNTCP guidelines.

The MSW made a total of four visits to the private practitioners. Initially, MSW collected data on KAP and after that sensitized the private practitioners by giving detailed explanation about case finding and referral of presumptive pulmonary tuberculosis cases to the Designated Microscopy Centre using the developed IEC material during third quarter (July–September 2017).

The MSW provided IEC poster to doctors for displaying at prominent places on the clinic walls. A case referral book was handed over to the practitioners. To keep a track of the cases, a copy of the case referral sheet was given by the PP's to the presumptive pulmonary tuberculosis cases. They were advised to hand it over at the identified nearest designated microscopy centers (DMC). The investigators regularly visited the Designated Microscopy Centre to validate the number of presumptive pulmonary tuberculosis cases referred by the practitioners. Compliance of the presumptive pulmonary tuberculosis cases to referral was assessed by calculating the

number of presumptive pulmonary tuberculosis cases who approached DMC with case referral forms out of the total who were referred by the practitioners. MSW made follow up visits to the private practitioner's clinic during the fourth quarter (October–December 2017) on a monthly basis for three consecutive months to determine the number of cases referred to DMC by private practitioners.

After the sensitization programme, improvement of case referral was assessed by comparing the number of cases referred by Private Practitioners to DMC in the third quarter with that of fourth quarter data maintained in the DMC records.

The data was entered in Microsoft excel and analyzed using SPSS 21 version. Demographic profile, knowledge, attitudes and practices of the practitioners as well as improvement in case finding and referral of presumptive pulmonary TB cases were described as frequencies and percentages.

3. Results

Our study had 34 private practitioners, most of them 20 (60%) were in the age group of 51–70 years, 27(79%) were males and 7(21%) were females. Majority (27) were general practitioners and 7 of them were specialists. Almost 13 doctors had more than 30 years of experience as private practitioners. Most of the private practitioners 23(67.6%) practiced at both morning and evening hours. About 19 doctors got less than 20 patients per day; only 1 doctor said out patients' strength was more than 40 per day(Table 1).

About 28 (82.4%) doctors were aware about Revised National TB Control Programme. The source of information for the 28 doctors were: 9 (32%) received information from internet, 8(28%) from Government hospital, 6(21%) from their colleagues, others from Indian Medical Association and medical journals respectively (Table 2). Almost all (97%) knew the causative organism of tuberculosis. Only half of the doctors were aware about the duration of cough as two weeks for presumptive TB cases. Only 19 (55.8%) private practitioners were aware about the first investigation of choice as sputum smear microscopy under RNTCP. About 16 (47%) of the doctors knew about the total number of sputum samples to be collected under RNTCP guidelines. Awareness about the ideal time of sputum collection was told correctly by only 8 doctors.

Table 3 states that overall the attitude of the PPs was good, except for one question where 38% were not sure and 6% of the PPs felt that "PP's should have the freedom to treat the patients on their own clinical decision and not by national program guidelines".

Table 4 depicts the practices adopted by the practitioners for TB case diagnosis and management. Approximately 74% of doctors advised the presumptive pulmonary TB cases to do sputum examination as first investigation of choice. Only 14 out of 34 doctors do sputum examination as follow up investigation of the TB patients, 6 of the PPs collected sputum samples in the OPD of which 50% of them sent the samples to private laboratories. Once they confirm the diagnosis as TB, 28

Table 1 – Socio-demographic profile of private practitioners.

Variable	Number (%)
Age in years	
31–40	3(8.8)
41–50	9(26.4)
51–60	10(29.4)
>60-70	12(35.3)
Gender	
Male	27(79.4)
Female	7(20.6)
Qualification	
General Practitioner	27(79.4)
Specialist	7(20.6)
Duration of service in years	
<10	6(8.8)
11–20 years	10(29.4)
21–30	5(14.7)
>30	13(38.2)
Timing of OPD	
Morning	9(26.5)
Evening	1(2.9)
Both	23(67.6)
24 hours	1(2.9)
Outpatient strength per day	
<20	19(55.9)
21–40	14(41.2)
>40	1(2.9)

Table 2 – Knowledge of private practitioners regarding RNTCP.

Variables	Number (%)
Awareness about RNTCP	
Yes	28(82.4)
No	6(17.6)
Source of information	
Government Hospital	8(23.5)
Medical Journal	2(5.9)
Indian Medical Association	3(8.8)
Internet	9(26.5)
Colleague	6(17.6)
Knowledge on causative organism	
Bacteria	33(97)
Virus	1(3)
Duration of Cough	
1 week	6(17.1)
2 weeks	18(52.9)
3 weeks	6(17.6)
4 weeks	4(11.7)
Investigation of Choice under RNTCP	
X-ray	12(35.2)
Sputum	19(55.8)
Culture	0
CBNAAT	1(2.9)
Tuberculin sensitivity test	2(5.9)
Number of sputum samples collected under RNTCP	
One	13(38.2)
Two	16(47)
Three	5(14.7)
Ideal time for sputum collection	
On spot	6(17.6)
Early Morning	19(55.8)
Both	8(23.5)
None	1(2.9)

Table 3 – Attitudes of the participants regarding RNTCP.

	Strongly agree	Agree	Not sure	Disagree	Strongly disagree
	No (%)	No (%)	No (%)	No (%)	No (%)
RNTCP training should be given to all private practitioners (PP)	11(32.4)	22(64.7)	1(2.9)	0	0
All TB patients should be referred to DOTS center	11(32.4)	22(64.7)	1(2.9)	0	0
Sputum of suspected TB cases should be examined only at Designated Microscopy Centers (DMC)	11(32.4)	15(44.1)	7(20.6)	1(2.9)	0
Anti-TB drugs to patients should be dispensed at all Medical stores	1(2.9)	2(5.9)	14(41.2)	14(41.2)	3(8.8)
There is a need for awareness on TB in the community	21(61.8)	12(35.2)	0	0	1(2.9)
PP's should have the freedom to treat the patients on their own clinical decision and not by national program guidelines	0	2(5.9)	13(38.2)	15(44.1)	4(11.8)

Table 4 – Practices of the practitioners regarding TB investigations and management.

Variables	Number (%)
First investigation in presumptive TB	
Correct practice	25(74)
Incorrect practice	9(26)
Place of referral for these investigations	
Correct practice	21(62)
Incorrect practice	13(38)
Investigation done for follow up TB cases	
Correct practice	21(62)
Incorrect practice	13(38)
Number of samples of sputum collected for diagnosing TB	
Correct practice	14(41)
Incorrect practice	20(59)
Management of TB case	
Correct practice	28(82)
Incorrect practice	6(18)
Number of practitioners treating TB cases	
Currently treating TB cases	3(8.8)
Not treating TB cases	31(91.2)

of them refer the patients to the government hospital for further management.

Only 6 (17.6%) PP's received training from the RNTCP. Out of 34 PP's, 24 of them received information from the PPM coordinator in their own clinics, out of which, only 16 doctors said they received information on case finding, referral and diagnosis. None of the PPs were registered under NIKSHAY.

The number of Presumptive pulmonary TB cases visited to DMC had improved from 14 to 34 (142%) and number of TB case detected from 3 to 5 (66%) (quarter before and after sensitization) (Table 5)

4. Discussion

It is one of the first study conducted to look at the strategy of involving MSW from medical college for strengthening RNTCP linkages at urban field practice area. The response rate was 94% which indicates that the private practitioners are open for collaborating with the medical colleges for RNTCP linkages.

Majority of them were senior practitioners over 50 years of age and most of them had more than 10 years of private practice. Though, they were aware about RNTCP, the etiology and symptoms of TB; there was a gap in knowledge regarding the necessary investigations to diagnose TB. More than half of the PPs knew that sputum examination was the investigation recommended by RNTCP, a big number 35% stated that radiological examination of chest as the recommended first investigation. A systematic review of 22 studies assessing health care provider knowledge about using sputum smears for diagnosis, 10 studies found that less than half of providers had correct knowledge with public sector providers having more knowledge than private providers.[7,8] A study among non-allopathic private practitioners found that only 14% could tell sputum examination to be the first investigation of choice in suspected TB cases.[9] A study done in Gwalior district and Hooghly district mentioned that 49% and 68% of PP's relied on chest radiology as first choice of investigation for diagnosing TB.[10,11]

In our study, 53% were not aware of the number of samples required for diagnosis and 76% of the participants were not aware about the time of sample collection. Similarly, in a West Bengal study only 43.3% and 33.3% replied correctly about number of sputum samples collected and timing of collection respectively.[12].This shows that TB diagnosis and treatment practices among private practitioners in India vary widely.[13] Only 18% had attended any training from RNTCP, 70.6% said that the RNTCP representative provided them with information. This reflects the need to reach out to the all the private practitioners and enhance their knowledge about RNTCP.

There are some gaps in the correct practices especially in investigations where 59% of the participants did not know how many sputum samples need to be collected and that 38% still referred the patients to private laboratories and not

Table 5 – Comparison of number of presumptive TB cases referred and number found positive before and after sensitization.

	Before sensitization	After sensitization	Increase in percentage (%)
Number of presumptive TB cases referred	14	34	142
Number of Cases found to be sputum smear positive	3	5	66

DMCs. Chirag et al stated that only 5% of new sputum positives were from the cases referred by PPs through PPM model.[14] However, in a study conducted in the southern states of India stated that 90% of PP's refer TB suspects to DMC.[15]

Even after a decade of RNTCP training to private providers, only 17.6% PP's received training from the RNTCP. Similarly, a study by Datta et al found that just over half of private providers reported exposure to RNTCP training.[16] About 82.4% of PP's in this study did not show willingness to be the part of the programme. In contrast Vaibhav et al in their study, about 82% of private practitioners expressed their willingness to be partners for implementation of national TB control programme.[17] This study thereby highlights the need to strengthen advocacy and communication strategies to enhance PP involvement in the program.

One of the objectives of the study was to assess the effectiveness of sensitization program in terms of number of cases referred to Designated Microscopy Centre by Private Practitioner. As mentioned by Sairu et al communication strategies like training and one-to-one dialogue with private practitioners may enhance TB notification.[18] In this study, after sensitization, the number of presumptive pulmonary cases in the fourth quarter, increased more than two folds when compared to previous quarter. A study conducted by Sachin Bhaskar Palve et al in Maharashtra, reported that diagnosis and referral for sputum diagnosis, categorization of treatment improved significantly after the sensitization workshop.[19]

The study had few limitations, firstly, the results cannot be generalized due to the limited sample size, secondly the non-allopathic practitioners that contribute to a large number of PPs in Bengaluru were excluded from the study, which we consider as a missed opportunity to collect data from this important group. Thirdly, the MSW from medical colleges were given meagre financial incentives to perform this task and the sustainability without incentives needs to be tested.

To conclude, the strategy of utilizing the services of medico-social workers employed in a medical college to sensitize and regularly co-ordinate with the private practitioners is feasible and promising. The involvement of practitioners has demonstrated the increase in number of presumptive TB case referrals to DMCs. The state of Karnataka with 56 medical colleges has ample scope to efficiently utilize this strategy to further strengthen the private practitioners involvement in RNTCP and their presumptive case referrals.

Disclaimer

The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions or policies of the State TB Office (RNTCP), Karnataka or the RNTCP State Task Force Operational Research Committee, Karnataka.

Authors contribution

S.Hemavarneshwari: Conception of idea, data collection, design, analysis, drafting of work.

Rizwana B Shaikh: Conception of idea, design, analysis, drafting of work.

Poonam R Naik: interpretation of results and revising draft.

Sharath Burugina Nagaraja: Design and revising critically for intellectual content.

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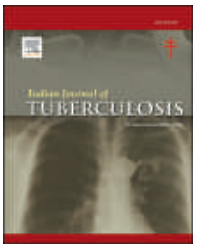

Original article

Tuberculosis related stigma attached to the adherence of Directly Observed Treatment Short Course (DOTS) in West Bengal, India

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ABSTRACT

Background: Stigma is a major barrier to the successful completion of the Directly Observed Treatment Short Course (DOTS). People put on DOTS have to face repeated exposure to stigma as per the requirement of the treatment. Thus stigma can shape the extent of access and adherence to treatment. But there is very little information available in Indian context explaining the extent of association between the stigma perceived among the patients and adherence to their DOTS therapy.

Aim: To explore the level of stigma perceived by the persons with TB and its influence on the adherence to DOTS treatment.

Methods: A cross sectional epidemiological study was conducted among 145 DOTS defaulters from three randomly selected districts in West Bengal. Respondents were approached at their households. Information was collected using a pretested questionnaire. Adherence to DOTS was grouped as early default (continued DOTS from 0 to 30 days) and late default (continued DOTS > 30). Stigma score was assessed using 11 item questions. Stigma score was grouped as low level (0–23) and high level (24–44). Analysis was done using Chi-square and multivariate logistic regression models to identify factors to influence adherence to DOTS. SPSS 23.0 version statistical software was used for analysis.

Results: Mean stigma score for the state was 23.0. Total 51 (40.69%) persons were within the low stigma group and 94 persons (59.31%) were within high stigma score group. District wise mean score was 19.8, 22.8 and 24.5 respectively for Birbhum, Jalpaiguri and North 24 Parganas. In North 24 Parganas, the high stigma score group accounted for 85.5% compared to 35.9% in Birbhum. Among the low stigma group, late default was 52.1% compared to 66.7% in high stigma group ($p = 0.054$). People with lower stigma level were 8.59 times more likely to have late default than the people with higher stigma level ($p = 0.001$).

Conclusion: Perceived stigma among the patients was identified as an important predictor for the adherence to DOTS therapy. Stigma reduction strategy should be designed to improve adherence to DOTS therapy. Present study recommends in-depth qualitative

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research to get more insight on the extent and shape of stigma and the way it influences the adherence. Apart from the stigma of the patients, influence of community stigma is a gray area for further research.

1. Introduction

The incidence of tuberculosis (TB) per 100,000 populations declined from 216 in 1990 to 167 in 2016.[1] In 2015, India contributed 25% of the global burden of tuberculosis (TB) incidence.[1,2] The Revised National Tuberculosis Control Program (RNTCP) emphasizes at three levels of care - (i) TB suspects are referred to the sputum testing facility under RNTCP, (ii) diagnosed cases are put on Directly Observed Treatment Short course (DOTS) treatment, and (iii) those who are put on DOTS, they must remain adherent to it.[3] As per the guidelines of RNTCP, the program puts efforts to mobilize the community towards public facilities as a core strategy.[4] But the public health system cannot adequately focus on social, psychological, cultural and political factors.[5,6] One of the most important barriers of satisfactory performance of RNTCP is the stigma attached to TB which remains as an under researched agenda in India.[3] In a study conducted in West Bengal, among highly stigmatized people, 20.5% respondents had delayed (14–25 days) and 79.5% had much delayed (26–120 days) seeking of sputum examination from public facility.[7] Stigma was an important delaying factor for sputum examination ($p < 0.01$).[8] Stigma attached to TB was a major barrier to access services in TB management.[9] Because of fear of infection, people often want to isolate the infected person from social functions, crowd or even restrict mixing up with family members.[10] Sometimes this was tied in with social capital when the person with TB did not have good.[11] People who are on DOTS have to come to the public health facility for observed treatment. In case of the sputum examination, a patient has to come to the facility twice. For DOTS treatment, the patient is repeatedly exposed to the facility and care providers and surrounding community. This leads to repeated exposure to stigma. The stigma can shape the extent of access and adherence to treatment.[11] There is very little information available in India about the effects of perceived stigma among the patients with TB and the community in the adherence of the DOTS treatment. Research will be relevant to find out level of perceived stigma and its effects on the adherence to DOTS. Under the limited scope of the present study in West Bengal, India research was conducted to measure the level of stigma perceived by people with TB infection and its influence on the adherence to DOTS treatment.

2. Methods

2.1. Study design and setting

We conducted a cross sectional epidemiological study. In West Bengal, there are 20 districts having 3 divisions – Jalpaiguri, Presidency and Bardhaman. From each division, one district was randomly selected. Thus, Birbhum, Jalpaiguri and North 24 Parganas were selected. As per the RNTCP, one Tuberculosis Unit (TU) caters 0.5 million population and one Designated Microscopy Centre (DMC) caters 0.1 million population.[3] From each district 4 TUs were selected using stratified sampling technique based on three criteria – population size, urban rural distribution and scheduled tribe (ST) population. Thus total 12 TUs were selected.

2.2. Sample size

The percentage of defaulter of DOTS was 7% in 2013 in West Bengal[12,13] that went up to 10% in many other states in 2014.[13] Using default rate as 10%, 10% absolute error, design effect 2; the sample size was calculated to be 70. The study had 145 defaulters to survey.

2.3. Selection of respondent

Respondents were approached at their household level. The DOTS register was maintained by DOTS Supervisor at each TU. The register was checked from 12 DOTS Supervisors at 12 TUs for last three years - on and before 30-June-2014. Around 4300 cases were identified who were put on DOTS. Only 149 defaulters were identified for the survey. From them, full information about 145 defaulters was collected based using a prefixed questionnaire. Information for each case was collected based on data recorded in the register and also through home visits being accompanied with the DOTS Supervisors.

2.4. Data collection

Information was collected using a pretested predesigned questionnaire that contained basic profile of the respondents and issues of stigma attached to TB. Stigma was assessed based on 11 question items adopted as per the tool of Moya used in 2010.[14] The survey tool had 1-4 options as per Likert scale. For 1, the respondent had strong disagreement; for 2, the respondent had disagreement; for 3, the respondent had agreement and for 4, the respondent had strong agreement. The tool and the statements for stigma measurement was field tested and changed from the feedback. The question was translated into Bengali version and the Bengali transcribed version was used for the interview. The Bengali version was field tested. The stigma section of the transcript containing 11 question items used for the assessment of composite stigma score had 82.3% sensitivity. Around 4.5% respondents could not understand 2–3 stigma question items clearly that required repetition and further explanation. It took around 45 minutes to complete each interview.

2.5. Statistical analysis

We used profile of the participants and their stigma level as two important research variables in our study. Assessment of stigma was estimated in 1–4 scale (1 – very little stigma, 2 – little stigma, 3 – moderate level of stigma, 4 – very high stigma). Using 11 item questionnaires, therefore, total score could be 11–44. Mean score was 22.8 and median was 23. Therefore, it was assumed that the low stigma range was 11–23, and the higher range was 24–44. We classified adherence to DOTS in two groups - early default, who defaulted within 30 days; and late default, who defaulted after 30 days.

SPSS statistics software version 20.00 was used for analysis. Apart from the basic descriptive statistics Chi-square analysis, the multiple logistic regression model was used to calculate Odds Ratio (OR) at 95% confidence level. We used multivariate logistic regression model to identify the most important predictors of stigma.

2.6. Ethical consideration

The ethical permission for human studies was obtained from all concerned authorities before commencement of the study. The Department of Health and Family Welfare, Government of West Bengal approved the study (vide memo HTB/31–2008/1054, dated 25-August-2012).

On the day of interviews, candidates who were ill or who did not consent to participate were excluded. For minors whose guardians were not present or refused to consent were also excluded from the study. From the 149 potential respondents, thus 145 respondents actually participated in the study.

2.7. Strength and limitation of the study

It is very much relevant to explore the effects of perceived stigma at the level of the patients and the community as large on the adherence to DOTS. Due to no funding available for this study, only stigma of the patients and its effect on DOTS adherence was explored. As per our literature review, in Indian context, it will be for the first time that the individual level stigma and its effect with the DOTS adherence have been measured.

3. Results

3.1. Characteristics of the participants

Among 145 respondents, three districts North 24 Parganas, Birbhum and Jalpaiguri contributed 42.8%, 26.8% and 30.4% respectively. Respondents of age groups 16–20 years and 20–40 years were 46.9% each. Males were 75.9%. Married respondents were 81.4%. Approximately 44.1% respondents were illiterate. Around 49.7% respondents were daily wage labors. We found that 68.3% respondents were slum dwellers and 45.5% reported to be in-migrant from Bangladesh, a neighboring country of India. Almost 70.3% respondents reported that they were users of any kind of substance like smokeless tobacco, cigar (branded or local), alcohol, brown sugar and other types of substances. Further detailed profile characteristics of the respondents are provided in Table 1.

3.2. Different aspects of stigma attached to the DOTS defaulters

Level of perceived stigma was assessed based on 11 question items adopted as per a standard tool[14] in 1–4 Likert scale. For 1, the respondent had strong disagreement; for 2, disagreement; for 3 agreement and for 4, strong agreement. In the following Table 2, item wise range of stigma has been provided for 11 statements.

3.3. Stigma score distribution among the DOTS defaulters

Because there was 11 such stigma items, we have provided distribution of perceived stigma for each item in 4 categories as described in section 3.2 before. This stigma score was calculated for each respondent. This could not provide a comprehensive and cumulative level of stigma of the entire respondent group. To measure this, average or mean of the stigma score of 145 respondents was calculated to be 22.8.

Total stigma score could be from 11 to 44. In the study group, mean stigma score was 22.8 and median was 23. Therefore, the stigma score was grouped as low level (11–23) and high level (24–44). Total 51 (40.69%) persons were within the low stigma group and 94 persons (59.31%) were within

Table 1 – Profile characteristics of the defaulters of DOTS.

Character	Group	N (%)	
Age group	16–20	9	(6.2)
	20–40	68	(46.9)
	40–60	68	(46.9)
Sex	Male	110	(75.9)
	Female	35	(24.1)
Marital Status	Married	118	(81.4)
	Single	27	(18.6)
	Others	0	(0.0)
Education	Illiterate	64	(44.1)
	Primary	35	(24.1)
	Upper primary	26	(17.9)
	Secondary	12	(8.3)
	Higher secondary and above	8	(5.5)
Caste	General	84	(57.9)
	Schedule Caste (SC)	37	(25.5)
	Scheduled Tribe (ST)	16	(11.0)
	Other Backward Class (OBC)	8	(5.5)
Religion	Hindu	118	(81.4)
	Muslim	27	(18.6)
Occupation	Govt. employee	23	(15.9)
	Private employee	48	(33.1)
	Contractual	2	(1.4)
	Daily wage labor	72	(49.7)
Slum dweller	Yes	99	(68.3)
	No	46	(31.7)
Migration status	Yes	66	(45.5)
	No	79	(54.5)
Substance use	Yes	102	(70.3)
	No	43	(29.7)

Table 2 – Different aspects of stigma attached to tuberculosis (showing adequate presence of stigma among respondents).

#	Statement of stigma issues attached to TB	Strongly disagree (%)	Disagree (%)	Agree (%)	Strongly agree (%)
1	I do not prefer a person with TB living in our community	36 (24.8)	52 (35.9)	44 (43.4)	13 (9.0)
2	I like to keep distance from people with TB	41 (28.3)	38 (26.2)	53 (36.6)	13 (9.0)
3	A person with TB is disgusting	37 (25.5)	62 (42.8)	39 (26.9)	7 (4.8)
4	I feel uncomfortable about being near those with TB	24 (16.6)	52 (35.9)	63 (43.4)	6 (4.1)
5	I do not want those with TB playing with our children	27 (18.6)	40 (27.6)	44 (30.3)	34 (23.4)
6	I do not want to talk to others with TB	41 (28.3)	55 (37.9)	43 (29.7)	6 (4.1)
7	I behaved differently toward a person with TB for the rest of his/her life	18 (12.4)	69 (47.6)	46 (31.7)	12 (8.3)
8	I may not want to eat with friends who have TB	36 (24.8)	43 (29.7)	53 (36.6)	13 (9.0)
9	I may not want to drink with friends who have TB	36 (24.1)	31 (21.4)	72 (49.7)	7 (4.8)
10	I usually try not to touch others with TB	35 (24.1)	70 (48.2)	34 (23.4)	6 (4.1)
11	Some people are afraid of those with TB	21 (14.5)	68 (46.9)	40 (27.6)	16 (11.0)

high stigma score group. District wise mean score was 19.8, 22.8 and 24.5 respectively for Birbhum, Jalpaiguri and North 24 Parganas. Distribution of low and high stigma score, and mean stigma score as proportion of maximum score (44) is depicted in Fig. 1 and Table 3.

We found concentrated default rate between 20 and 60 days at the stigma score ranges of 12–18 and 25 to 32. Please refer to Fig. 2.

We calculated stigma score in three districts to see that in North 24 Parganas, the high stigma score group counted maximum (85.5%). Further details are provided in Table 3.

3.3.1. *Stigma in urban and rural area*

High stigma score was found in the rural area (60.9%) compared to the urban area (53.8%). It is depicted in Table 4.

3.4. *Factors influencing the level of stigma*

We categorized default in two groups - early (default within 30 days from the start of DOTS) and late default (default after 30 days). We wanted to explore whether delay in default was associated with stigma. It was found that late default was 52.1% in the low stigma group compared to 66.7% in high stigma group ($p = 0.054$). Please refer to Table 5.

We did multivariate logistic regression analysis to identify that stigma was the most forceful factor to influence the delay of DOTS default. People with lower stigma level were 8.59 times more likely to have late default than the people with higher stigma level. In other words, people with lower stigma were 8.59 times more likely to remain adhered to DOTs than those who had higher level of stigma. Please refer to Table 6.

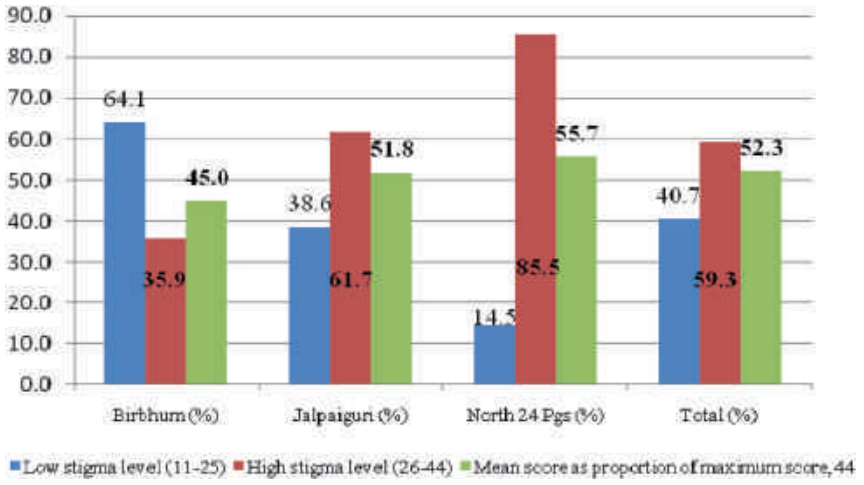

Fig. 1 – Distribution of low and high stigma scores among three districts and their mean scores (showing Birbhum as lowest stigma and North 24 Parganas as highest).

Table 3 – Stigma level among the respondents in three districts (showing higher stigma level in North 24 Parganas compared with Birbhum and Jalpaiguri).

Item	Birbhum (%)	Jalpaiguri (%)	North 24 Parganas (%)	Total (%)
Low stigma level (11–23)	25 (64.1)	17 (38.6)	9 (14.5)	51 (40.7)
High stigma level (24–44)	14 (35.9)	27 (61.7)	53 (85.5)	94 (59.3)
Mean score (% of 44)	19.8 (45.0)	22.8 (51.8)	24.5 (55.7)	22.8 (52.3)

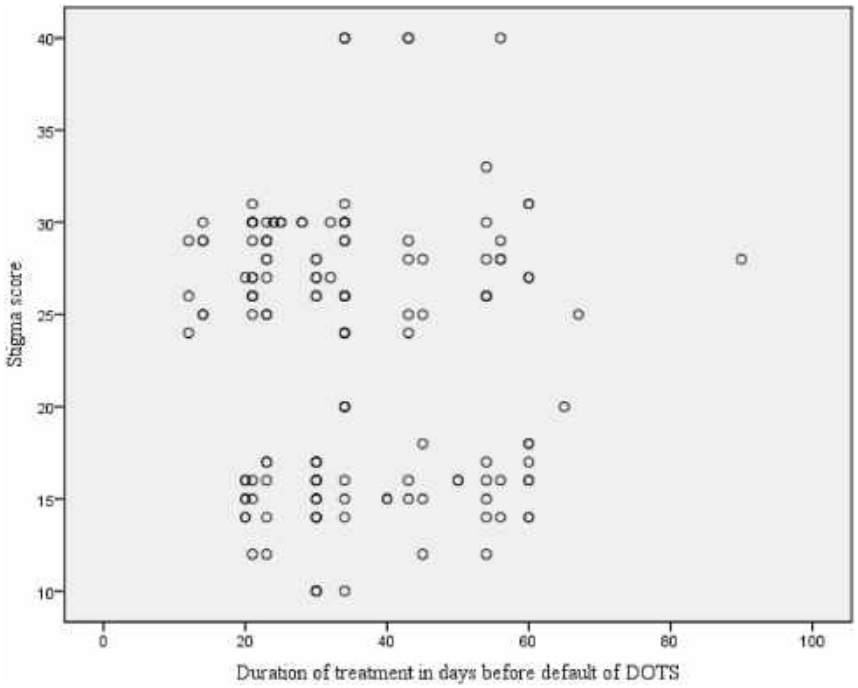

Fig. 2 – Scatter diagram of the distribution between stigma score and duration of treatment of DOTS (showing concentrated default rate between 20 and 60 days at the stigma score ranges of 12–18 and 25 to 32).

Apart from stigma, we found some other factors to influence adherence to DOTS. They are caste [ST: OR 7.667 (2.690–20.820, p = 0.011)], type of labor [migrant: OR 0.116 (0.032–0.424), p = 0.001] and knowledge about tuberculosis [knowledge: OR 0.055 (0.006–0.513), p = 0.011].

4. Discussion

Percentage of people defaulted from DOTS was around 7% in 2011, 2012 and 2013 in West Bengal. Number of Multi Drug Resistant TB was in rising trend since last few years in West Bengal.[13] This is related to default from DOTS and our current study in this background, explored factors behind default. In our study, it was observed that stigma (OR- 8.594: 2.405–30.703: p = 0.001) was found as a very important factor to influence the adherence of DOTS treatment. Rate of default from DOTS in West Bengal was 7% compared with 6% in India.[15] Based on an estimation, the state of West Bengal is losing opportunity to cure 1023 TB cases per year.[13,15] Regarding stigma, study findings have implications for (1) informing health policy decision makers about how persons are affected with TB and interpret their TB illness and stigma attached to it and secondly, (2) research on health related stigma, and (3) expanding structural stigma reduction interventions.[14] In a study in North 24 Parganas in West Bengal in 2015, stigma influenced the adherence to DOTS.[16] The scatter diagram in our study (Fig. 2) reflects the relationship between stigma and duration of adherence to DOTS.

We calculated inter-district variations of stigma and early default rates among Birbhum, Jalpaiguri and north 24 Parganas. It was found that in Birbhum proportion of respondents with high stigma was least, but early default rate was highest. This needed further research. Our study had limitations to conduct further in-depth analysis to put more insights into its cause. In Birbhum, compared with North 24 Parganas, proportion of tribal population was much higher. The role of these excluded groups remains an area of further research. Many socially excluded patients were at risk of delayed presentation, poor adherence, and loss to follow-up.[17] In our study, scheduled caste was found to be important factor to influence adherence to DOTS (OR-7.667: 2.690–20.820, p = 0.011) as well. Study further recommends conducting in-depth qualitative studies to get more insight into the cause of exclusions from DOTS in the context of stigma. Research on

Table 4 – Stigma level among the respondents in urban and rural areas (showing higher stigma level in rural area).

Level of stigma	Urban	Rural	Total
Low stigma (11–23)	49 (49.5)	18 (39.1)	67 (46.2)
High stigma (24–44)	50 (50.5)	28 (60.9)	78 (53.8)
Total (11–44)	99 (100.0)	46 (100.0)	145 (100.0)

Table 5 – Percentage distribution of period of adherence.

	Early default of DOTS (up to 30 days)	Late default of DOTS (>30 days)	p
	N (%)	N (%)	
Stigma score			
Low (11–23)	45 (47.9)	49 (52.1)	0.054
High (24–44)	17 (33.3)	34 (66.7)	

Table 6 – Strength of association between the level of stigma and the delay in the default of DOTS, adjusted for several other factors (showing that stigma is the most powerful factor).

Item	Multivariate logistic regression analysis (OR of early and late DOTS defaulter)			
	P	Adjusted OR	95% C.I. of OR	
			Lower	Upper
Male	0.836	0.784	0.078	7.877
Female				
Illiterate	0.413	0.278	0.013	5.961
Primary	0.413	0.282	0.014	5.845
Upper Primary	0.248	0.149	0.006	3.776
Secondary	0.283	0.133	0.003	5.282
Higher Secondary & above				
General	0.474	2.471	0.208	29.363
SC	0.228	5.257	0.354	78.085
ST	0.011	7.667	2.690	20.820
OBC				
Hindu	0.700	0.707	0.121	4.127
Muslim				
Employed, Government	0.599	1.607	0.274	9.439
Employed, private	0.626	1.422	0.346	5.852
Daily wage labor		2.345	3.467	13.453
Others				
APL	0.726	1.267	0.337	4.768
BPL				
Urban	0.222	3.156	0.500	19.928
Rural				
Dweller in slum, Yes	0.087	4.630	0.801	26.752
Dweller in slum, No				
Migrant, Yes	0.001	0.116	0.032	0.424
Migrant, No				
Substance use, Yes	0.104	0.261	0.052	1.321
Substance use, No				
Age group 0–20 yrs	0.898	0.801	0.027	23.860
Age group 21–40 yrs	0.996	1.003	0.363	2.771
Age group 41–60 yrs				
Knowledge score Low (0–2)	0.011	0.055	0.006	0.513
Knowledge score High (3–6)				
Stigma score Low (11 30)	0.001	8.594	2.405	30.703
Stigma score High (31 44)				

the causes and extent of stigma will be useful to guide health and social interventions that reduce its effects. Also there is importance of research that focuses on the behavioral, psychological as well as in the social context and dimensions of TB-related stigma.[14]

Our study could also identify a few other factors like migration (OR 0.116: 0.032–0.424; p = 0.001) and level of knowledge about TB (OR 8.594: 2.405–30.703, p = 0.011) to influence the duration of adherence to DOTS. Knowledge of TB was also associated significantly with the level of stigma (p = 0.011). Our study therefore could open new avenues of research on tuberculosis program to make it more effective in the context of better adherence to DOTS regime.

5. Conclusion

The study could provide insights into an under researched area. It has evidenced the negative influence of stigma in the adherence of DOTS. Apart from stigma; migration, caste and knowledge about TB are also found to be influencers of adherence to DOTS. Stigma is found as a constant barrier. Stigma reduction strategy should be designed to improve adherence to DOTS therapy from the experiences of successful experiments done elsewhere, for example in Thailand. Stigma reduction in the community is a pivotal step towards improved DOTS adherence. This was not the scope of this study. Therefore, apart from the stigma of the patients, influence of community stigma is a gray area for further research. The TB control program in West Bengal should also give priority on the issues like migration and caste. Migrants face lot of hindrances to remain adhered to treatment. Flexibility of DOTS regime and certain innovative strategies may solve their problems. People from backward caste are hesitant to access healthcare services. In-depth studies may be conducted to explore the roles of migration and caste in the adherence of DOTS regime and their extent.

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Conflicts of interest

All 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.2019.03.005.

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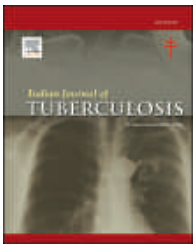

Review article

The goal to end TB- strategy: Public private partnership. Is it only private sector to blame?

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ABSTRACT

National Strategic Plan for tuberculosis care and control, Nepal (2016-2021) envisions ambitious target of decreasing TB incidence by 20% by 2021.In achieving so it has to identify and manage 20,000 more cases by 2021 compared to 2015. Contribution of private sector which roughly accounts to 25% of all the burden of tuberculosis cases in Nepal will be vitally important along with effective public private partnership. In this review we discuss why blaming the private sector alone is not sufficient and much effective PPP collaboration needs to be done stepping on the positive results shown by the earlier collaboration.

WHO estimates 45,000 new cases of tuberculosis every year in Nepal with estimated incidence rate of 152/100,000 population.[1,2] However, the recorded cases of 31,764 as of year 2017, highlights there are still plenty number of cases missing.[2] Furthermore, there is an increasing concern on the rise in number of cases of Drug Resistant tuberculosis. As of National survey done in 2011, DR TB among the new TB patients is 2.2% while among the previously treated cases is at 15%.[2] While the accurate estimate of tuberculosis burden possibly will be given by prevalence survey, which has just been recently started in 2018, question remains about the steep decline in number of cases over the years.

Case holding by Doctors, particularly working in the private sectors, has been linked as one of the attribution to those missing cases and not notified in the Government system. This has been of similar concern in neighboring country India[3] where larger issue of multi drug resistant tuberculosis also remains. One of the study done from drug sales data estimated roughly 2.2 million cases to be privately treated in India, which was found more than twice fold increased than actually assumed.[4] Nepal is in process of endorsing mandatory notification of tuberculosis. Evidence suggests mandatory notification has had positive impact on case notification in various countries.[5]

The effectiveness of Directly Observed Treatment Short course (DOTS) has been very well documented. With time, DOTS has evolved itself from clinic based to community based to family based depending on country and context with variable successes.[6]Higher success rate from the DOTS has been very well documented in number of studies.[7] Even in Nepal the success rate of DOTS is largely maintained around 90%. Despite these, a larger faction of private doctors are not sending the patients to the DOTS which is available free of cost in government health facilities.

Barrier to referral has been identified at both the health system and the patient perspective. Long distance time,

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inconvenient opening hours, attitude of the health workers in public places, stigma and discrimination associated with the disease along with cumbersome process for patients to follow up with DOTS losing their daily wages can be possible factors.[8–10] These are only foreseeable factors not to forget the other dimensions such as the patient factors and specific health service factors. Furthermore, poor public private partnership, limited availability of modern diagnostic in the country (Gene-Xpert) further complicates the scenario.

The importance of public private partnership for Tuberculosis control program has been long written. Even in Nepal the importance of PPM is well understood. Majority of the initiatives in the past were project bound with time dependent, limited to specific geographic area and limited providers. However strong commitment from the National Tuberculosis Control Program is now needed to give proper stability to the PPM strategy. Evidence suggest considerable impact of PPM strategy in case notification along with success rate when implied strongly by involving the private practitioner in Nepal.[11] When done with strong commitment private sector can be involved into the National TB control Program.

It is now time for the NTP to value the evidence from previous research which were project based and translate into visible policy to bring the private sector into the mainstream NTP. Establishment of PPM taskforce at different levels under federal structure (central, provincial and local) with strong monitoring mechanism could be the first step in the process. Secondly, initial involvement of key private practitioners involved in the TB diagnosis and treatment can be done. This will help in bringing other private practitioners in the TB control program. Thirdly, strong regulatory mechanism for ATT drugs in the market with mechanism to ensure for completion of treatment needs to be in place and timely quality assessment of the involved private sectors needs to be done. Finally, to motivate the private sectors in the TB control program performance-based financing has to be implemented. These initiatives are only few measures to bring the missing number of TB cases in mainstream NTP, as failure to timely address the private sector will further continue to increase the gap remaining in the country.

Tuberculosis was, Tuberculosis is and with emergence of MDR and XDR strains it will possibly continue to be a killer disease. This applies even more for country like Nepal with limited resource at disposal to tackle the resistant strain. It is imperative that the National TB control program learn from it's past experience of Urban TB control Program in bringing the private sector in a holistic way to address the missing estimated cases.

Conflicts of interest

The author has none to declare.

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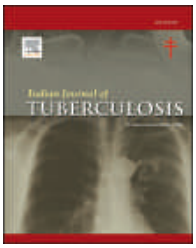

Review article

Evidence based interventions and implementation gaps in control of tuberculosis: A systematic review in low and middle-income countries with special focus on India

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ABSTRACT

We synthesised the findings of intervention studies on Tuberculosis control (TC) in low- and middle-income countries with specific reference to India through a systematic review during the period 2000–2017 in order to identify the implementation gap. The research questions were framed using PICOS (population, intervention, comparison, outcomes and study design) framework and PRISMA (preferred reporting items for systematic reviews and meta-analyses) guidelines were used for study selection. The search was mainly carried out in MEDLINE/PubMed, Web of Knowledge and Cochrane libraries. DOTS was found to be the most effective intervention program for control of Tuberculosis. Lack of utilization of the capacity of various level health staff, accessibility in utilizing health facilities and insufficient community involvement was identified as the major gaps for TC. In the case of India, each state has its own priority and applicability for different TC interventions. Most of the studies on implementation of the TC program supported the encouraging effect of the intervention in the control of Tuberculosis. The specific need of each country is clearly reflected in many of the selected studies. In order to establish the association of intervention and its implementation gaps on TB control, more rigorous evaluation methods are needed including meta-analysis.

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1. Background

Tuberculosis (TB) is the ninth leading cause of death in the world.[1] In 2016, among the 10.4 million people with TB and 1.7 million deaths from TB globally, over 95% of TB deaths and cases were in low- and middle-income countries (LMIC). Seven countries accounted for 64% of the total TB cases with highest percentage seen in India followed by Indonesia, China, Philippines, Pakistan, Nigeria, and South Africa.[2] Both World Health Organization's (WHO) End TB Strategy and the United Nations' (UN) Sustainable Development Goals (SDGs) have a common aim to end the global TB epidemic.[3]

India is on the top of first five countries, with 56% of estimated cases of TB in the world.[4] The country accounted for 33% of global TB deaths among HIV-negative people, and for 26% of the combined total TB deaths in HIV-negative and HIV-positive people.[4] Globally the highest burden of both TB and multi-drug-resistant TB (MDR-TB) is reported to be in India.[4] India accounted for an estimated incidence of 2.79 million cases of TB in 2016.[5] In India, TB treatment and care are being provided in the government's Revised National TB Control Programme (RNTCP). TB treatment is also provided through private sector health providers in the country. RNTCP is one of the vertical programs for TB control that have been initiated by the government in 1997. Now RNTCP has covered all over India, making it the second largest such program in the world.[6] In the five-year National Strategic Plan for 2012–2017, TB free India was the vision of the government through achieving universal access. Implementation of the national strategic plan (NSP) was one of the main areas of RNTCP in this period. In spite of the remarkable achievement of NSP, people seeking care from private sector is a big challenge.[7,8] So, in NSP 2017–2025, participation of private sector was integrated into the plan in order to ensure quality care and treatment. Now, Government of India announced the elimination (as defined by the WHO, there should be less than one case of TB for a population of a million people) of TB by 2025.

TB is a curable and preventable disease. Hence, most deaths from TB could be prevented with early diagnosis and appropriate treatment. So, the interventions in the area of TB control needs special importance as it is the most effective way to control this infectious disease. With this background, this review attempts to synthesise the findings of intervention studies carried out on TB control in LMICs with specific reference to India through a systematic review of literature during the period 2000–2017 from an implementation gap perspective Table 1. This article seeks to address the following review questions:

1. What are the published available interventions on control of TB in LMICs including India?
2. What are the gaps identified in implementation on the intervention for the TB control programs in India?

2. Methods

We followed the five-step strategy for conducting this systematic review similar to the one proposed by Khan K et al 2003.[9] The first step to frame questions for the review was done by all the three authors jointly. In the second step the second author identified the relevant work related to the present study with the help of a research assistant. The quality of the studies based on the objective of this review was assessed by all the authors and we summarized the evidences from the selected studies based on the research question. Narrative approach of data synthesis was adopted. Finally, based on the findings of the studies, conclusions were drawn. The study has been registered in PROSPERO (reg no CRD42018070406).

3. Search strategy

3.1. *Study design and data sources*

We systematically reviewed literature published during June 2000 to June 2017 to identify studies on interventions for control of tuberculosis and evaluated the implementation gaps. Our priority was to select studies from LMICs including India. A comprehensive search strategy was used with suitable key words used to identify pertinent literature. The search was carried out in MEDLINE/PubMed, Web of Knowledge and Cochrane libraries. Additional studies were identified by manual search and cross referencing.

A detailed search strategy was executed. The following key words were used: "Tuberculosis", "implementation gaps", "control programs", "RNTCP", "policy evaluation", "evidence-based policies", "implementation science", "interventions". Key words were combined using Booleanoperators and the database specific controlled vocabulary.

3.2. *Methodological quality appraisal*

The methodological quality or risk of bias of the studies included were not appraised in consistent with guidance.

3.3. *Selection criteria*

Based on the objective of the study, inclusion criteria were fixed a priori by the authors. Original full text articles published in English in the above search engines in this particular period were included. As per the inclusion criteria, articles on intervention studies on control of TB and gaps identified in implementation of the intervention programs were included. Studies reporting prevalence and correlates of TB were excluded (Table 2).

3.4. *Data extraction and analysis*

All search results were entered into Zotero and duplicates were removed. The authors reviewed titles and abstracts in order to ensure the selection criteria matching with our objective. A total of 1324 records were screened, and 77 full text articles were extracted and reviewed independently by two authors. Discrepancies in the decisions were resolved with the aid of a third reviewer. For inclusion and exclusion criteria we followed preferred reporting items for systematic reviews and meta-analysis

Table 1 – Final search terms used with PICOS framework.

Population	Intervention/Comparator	Outcome
Adults population with tuberculosis in Low and middle-income countries	All interventions targeting control of TB	Major interventions for TB control Major implementation gaps in TB control interventions

PICOS-patient/problem/population, intervention, comparison/control/comparator, outcomes, study type.

Table 2 – Inclusion and exclusion criteria.

PICOS	Inclusion criteria	Exclusion criteria
P	Individuals from LMICs with Tuberculosis	Prevalence of TB Correlates of TB
I	All interventions	National TB control programs by the governments
C	Interventions and implementation gaps	Government documents
O	Intervention gaps	Government document
S	All study types including quantitative and qualitative studies, systematic reviews, meta-analysis etc	National TB control program evaluation

PICOS-patient/problem/population, intervention, comparison/control/comparator, outcomes, study type.

(PRISMA) guidelines (See Fig. 1). Of this, 29 articles met our inclusion criteria. From these we categorized the articles into interventions and program implementations. All the articles selected were reviewed by all the authors. Table 3 describes the characteristics of the articles selected for the final review.

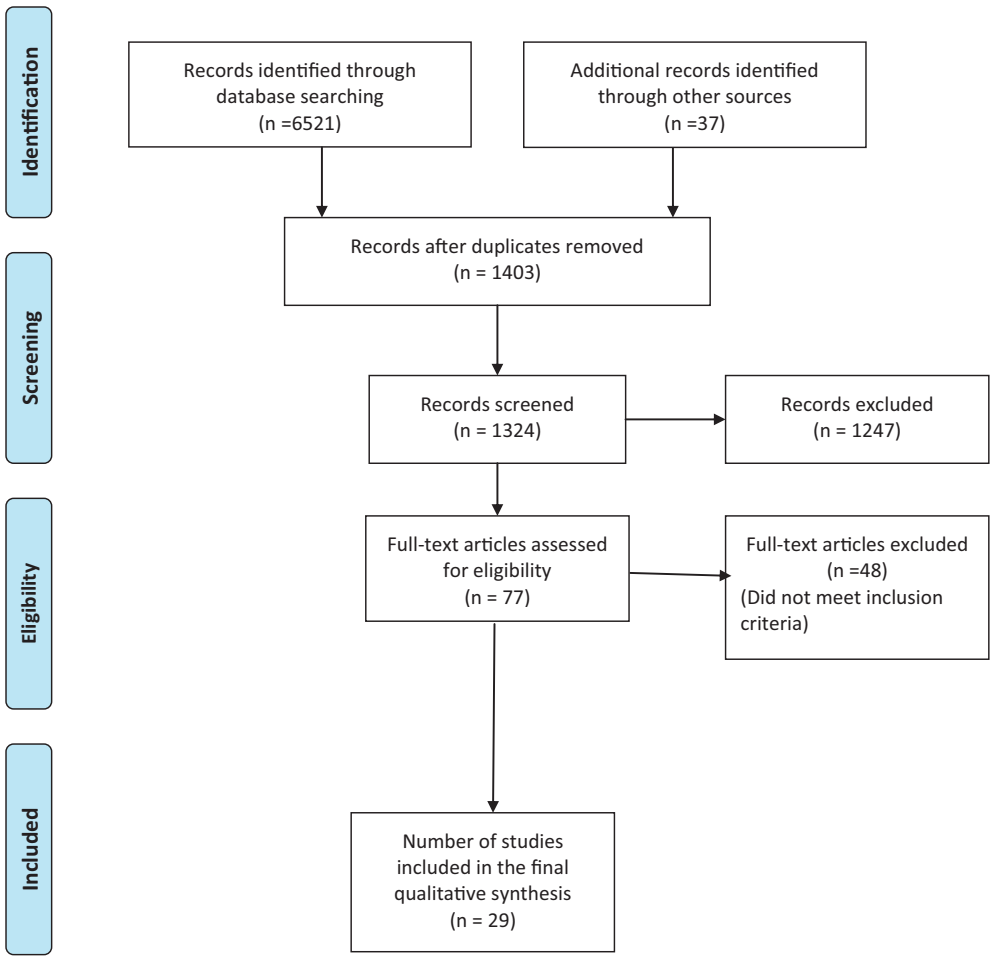

Fig. 1 – Prisma flow diagram.

Table 3 – Details of included articles: Studies on Intervention and implementation gaps studies.

Ref No	Author & year	Title of the study & Journal	Country	Type of study
Studies on Intervention				
10	Wingfiel T et al, 2015	Designing and implementing a socioeconomic intervention to enhance TB control: operational evidence from the CRESIPT project in Peru; BMC Public Health	Peru	Randomized Controlled Trial
11	Tola HH et al, 2016	Psychological and Educational Intervention to improve Tuberculosis Treatment Adherence in Ethiopia Based on Health Belief Model: A cluster Randomized Control Trial; PLOS ONE	Ethiopia	Randomized Controlled Trial
12	Colvin C et al, 2014	Evaluation of community-based interventions to improve TB case detection in a rural district of Tanzania; Global health: Science and Practice	Tanzania	Cross sectional pre-post evaluation
13	Kironde S et al, 2002	Community participation in primary health care (PHC) programmes: Lessons from tuberculosis treatment delivery in South Africa; African Health Sciences	South Africa	Prospective quantitative study
14	Tulloch O et al, 2015	Patient and community experiences of tuberculosis diagnosis and care within a community-based intervention in Ethiopia: a qualitative study; BMC Public Health	Ethiopia	Qualitative
15	Ollé-Goig JE et al, 2001	Treatment of tuberculosis in a rural area of Haiti directly observed and non-observed regimens. The experience of Hospital Schweitzer; The International Journal of Tuberculosis and Lung Disease	Haiti	Retrospective study based on clinical records
16	Das M et al, 2014	Directly-Observed and Self-Administered Tuberculosis Treatment in a Chronic, Low-Intensity Conflict Setting in India; PLOS ONE	India	Retrospective observational cohort study
17	Heller RF et al, 2006	Prioritising between direct observation of therapy and case-finding interventions for tuberculosis: use of population impact measures; BMC Medicine	India	Comparative study of two interventions
18	Frieden TR et al, 2003	Impact of national consultants on successful expansion of effective tuberculosis control in India; The International Journal of Tuberculosis and Lung Disease	India	Pre-post evaluation using medical records

(*continued on next page*)

Table 3 – (*continued*)

Ref No	Author & year	Title of the study & Journal	Country	Type of study
19	Venugopal K et al, 2008	Treatment Outcome of Neuro Tuberculosis Patients Put on Dots-an Observation Study from the Field; Indian Journal of Tuberculosis.	India	Hospital based cohort study
20	Hadley M et al, 2000	Community involvement in tuberculosis control: lessons from other health care programmes; TheInternational Journal of Tuberculosis and Lung Disease	Developing countries	Review
21	Borgdorff MW et al, 2002	Interventions to reduce tuberculosis mortality and transmission in low-and middle-income countries; Bulletin of World Health Organization	LMIC	Literature Review
22	Arshad A et al, 2014	community based interventions for the prevention and control of tuberculosis; Infectious diseases of Poverty	NA	Systematic Review
23	Adams LV et al, 2014	Interventions to improve delivery of isoniazid preventive therapy: an overview of systematic reviews; BMC Infectious Diseases	NA	Systematic Review
24	Cobelens F et al, 2012	Research on implementation of interventions in Tuberculosis control in Low-and middle-income countries: A systematic review; PLOS Medicine	LMIC	Systematic Review
25	Jeyashree K, e al, 2016	Smoking cessation interventions for pulmonary tuberculosis treatment outcomes (Review); Cochrane Review	NA	Cochrane Review
26	Awaisu A et al, 2011	The SCIDOTS Project: Evidence of Benefits of an Integrated Tobacco Cessation Intervention in Tuberculosis Care on Treatment Outcomes; Substance Abuse Treatment, Prevention and Policy	Malaysia	Quasi experimental
Studies on implementation gaps				Country
27	Essa SA et al, 2017	Assessment of the participation of primary care services in national tuberculosis control program in Gharbia Governorate; Egyptian Journal of Chest Diseases and Tuberculosis		Gharbia Governorate
28	Khan AH, 2017	Tuberculosis Control in Sindh, Pakistan: Critical Analysis of its Implementation; Journal of Infection and Public Health		Pakistan
29	Seddiq K et al, 2014	Implementing a successful tuberculosis programme within primary care services in a conflict area using the stop TB Strategy: Afghanistan case study; Conflict and Health		Afghanistan
30	Roy TK et al, 2015	Bridging Gaps in Revised National Tuberculosis Control Program at Bankura District, West Bengal State, India; American Journal of Public Health Research		India

31	Mabunda JT et al, 2016	Needs assessment for adapting TB directly observed treatment intervention program in Limpopo Province, South Africa: A community-based participatory research approach; African Journal of Primary Health care & Family Medicine	Africa
32	Fiseha D and Demissie M, 2015	Assessment of Directly Observed Therapy (DOT) following tuberculosis regimen change in Addis Ababa, Ethiopia: a qualitative study; BMC Infectious Diseases	Ethiopia
33	Rensburg HCJ et al, 2004	Social Research as an Intervention Tool in Tuberculosis Control; The International Journal of Tuberculosis and Lung Disease	South Africa
34	Yassin MA et al, 2006	Ten-year experience of the tuberculosis control programme in the southern region of Ethiopia; The International Journal of Tuberculosis and Lung Disease	Ethiopia
35	Khatri GR and Frieden TR, 2002	Rapid DOTS Expansion in India; Bulletin of the World Health Organization	India
36	Waisbord S,2010	Participatory Communication for Tuberculosis Control in Prisons in Bolivia, Ecuador and Paraguay; Rev PanamSaludPublica	Bolivia, Ecuador and Paraguay
37	Hou WL et al, 2012	Implementation and community involvement in DOTS strategy: a systematic review of studies in China; The International Journal of Tuberculosis and Lung Disease	Systematic Reviews
38	Toczek A et al, 2012	Strategies for reducing treatment default in drug-resistant tuberculosis: systematic review and meta-analysis; The International Journal of Tuberculosis and Lung Disease	Systematic Review

NA: Not Applicable, LMIC: Low and middle-income countries.

4. Results

4.1. *Study selection*

Finally, we identified a total of 29 original articles for final synthesis. In this manuscript, the synthesis is presented theme wise into two: studies on interventions in TB control programs and studies on implementation gaps. There were 17 studies in the first category including five systematic reviews and 12 studies in the second category including two systematic reviews.

4.2. *Studies on interventions in TB control programs*

The review in this section rests on interventions in TB control across LMICs. We used WHO criteria for the selection of LMICs. The following section provides the main findings of the selected intervention studies.

4.2.1. *Randomized Controlled Trials*

Two were Randomized Controlled Trials (RCTs):one from Peru[10] and the other from Ethiopia.[11] Socio-economic intervention was delivered along with the national TB control program to the intervention group against the normal standard of care from the National TB program in a study from Peru as part of a large project on Community Randomized Evaluation of a Socioeconomic Intervention to Prevent TB (CRESIPT). The economic support included cash transfer to patient's households. Social interventions were household visit, participatory community meetings, mutual support including stigma reduction and community empowerment. Even with logistic difficulties, socio-economic intervention played a major role in control of TB in similar settings. The study from Ethiopia was a hospital based RCT conducted among 698 TB patients based on health belief model. The four months intervention included several sessions of psychological counselling and educational intervention. The pre-post evaluation findings indicated significant improvement in the intervention group with regard to adherence level compared to control group.

4.2.2. *Community based studies*

A cross sectional survey was conducted in Tanzania to determine effectiveness of community-based intervention on TB case detection. Sensitizing regional and district TB co-ordinators, community leaders, and community-based organizations, training pharmacists and traditional healers, training, deployment, and supervision of two sputum fixers and training to eight current TB patients were done as part of the intervention.[12] Two years after the intervention, the case notification rate for smear-positive TB increased by 68%. The findings of the study indicated the importance of community-based interventions on improved TB case notification.

The primary focus of a community-based study in South Africa was the feasibility of community participation in a high burden TB program in a resource limited setting.[13] The adult TB patients in this study were given options to choose community-based treatment, clinic-based treatment and self-administered treatment. Volunteers supervised the patients

who opted community-based Directly Observed Therapy (DOT). The study established the important role of lay volunteers in TB treatment supervision compared to other methods of TB treatment delivery. After a year, no significant difference was seen between the treatment options regarding the outcome among new patients. However, for re-treatment of patients, community-based supervision was found to be superior to self-therapy. A high level of female patient participation was another major finding of the study.

A qualitative study was reported from Ethiopia on the community members treatment seeking behaviour and their perceptions of the intervention based on 36 in-depth interviews with TB patients who were on treatment or screened negative for TB.[14] A community intervention as part of the Ethiopia Health Extension Program appointed female health extension workers (HEW) and volunteer Community Health Promoters (CHP). These workers were giving advocacy, communication and social mobilization activities identifying symptomatic individuals, collecting sputum and preparing smears at community level. The intervention was very much acceptable, especially for poor men and women and those who had difficulty in traveling. A high level of appreciation was seen in case of giving sputum samples and receiving results from within their home communities.

A retrospective study based on clinical records in Haiti compared two strategies for TB treatment: usual standard treatment regime and supervision by former TB patients at home for DOTS (DOT group) and non-observed treatment (non-DOT group).[15] The area was known for its extreme poverty and high rates of HIV infection. Medication together with free food for a complete one-day diet and fuels for cooking were brought to the patients home twice weekly for DOTS group along with former TB patient supervision. For non-dots only, medicine was given. The successful outcome was significantly higher for DOTS than non-DOTS (87% vs 58%) and this difference was also seen among HIV infected patients.

4.2.3. *Indian studies*

Among the four studies from India, one was a retrospective observational cohort study which compared two treatment methods: DOTs and SAT (self -administered therapy).[16] The study was conducted in the border of Chhattisgarh, Andhra Pradesh and Odisha states in India. Because of the conflict situation in these regions, the accessibility to primary and secondary health care was poor. The success rate was 69% among those who treated under intermittent DOTs compared to 53% under SAT. The duration of treatment for patients under DOTs was shorter than under SAT. Though not superior, SAT can be a feasible option to be adopted in conflict situations where as DOTs is difficult to be implemented.

A study by Heller et al estimated the population impact measures of two interventions using published literature based on national data.[17] The study compared the direct observation of therapy and increasing case-finding. The study estimated that increasing case finding for TB saves nearly 10 times more lives than with the use of the directly observed component of DOTS in India.

Another study was on the impact of national consultants on TB control programs. The study compared areas with and without consultants and individual areas before and after consultation. The study finding indicated that DOTs delivery as well as treatment success was significantly higher in areas with medical consultants. However, the study identified that there were so many difficulties in appointing consultants in all the areas.[18]

The research study conducted in the Indian state of Kerala assessed effectiveness of Revised National TB Control Program among adult neuro tuberculosis patients.[19] After the routine RNTCP program monitoring along with the supervision of drug intake a treatment completion rate of 81% was seen.

4.2.4. *Literature reviews*

Six selected studies were literature reviews. Among them three were systematic reviews; one Cochrane review and two studies on literature review on LMICs.

Hadley and Maher in 2000 published a review on community-based health care initiatives for TB control in developing countries.[20] The main areas identified were informal community involvement like delivery of messages to encourage TB suspects to encourage treatment, psychological and logistic support to complete the treatment and in case of formal community involvement like ensuring programs for accessibility of treatment by disseminating messages to increase awareness and encourage adherence, identifying the following those who break treatment, identify adverse effect and case detection.

A literature review on interventions to reduce TB mortality and transmission in LMICs specified the available TB interventions as part of the TB control programs such as diagnosis and treatment of smear-positive tuberculosis, BCG immunization, diagnosis and treatment of smear-negative tuberculosis, active case finding and treatment of smear positive tuberculosis, preventive therapy in people with HIV infection and preventive therapy for contacts of tuberculosis patients and adults in the general population. Amongst these, DOTS was found to be the most cost-effective intervention.[21]

A systematic review using 41 studies on community-based interventions for the prevention and control of TB reported that community-based interventions showed significant increase with risk ratio 3.1 in TB detection rates and a non-significant impact on TB incidence.[22] Treatment success rate was also high in case of community-based interventions and the involvement of community health workers further improved access and service utilization.

In order to compare different organizational interventions to improve isoniazid preventive therapy (IPT), a systematic review was conducted by Adams et al in 2014.[23] IPT delivery was measured by treatment completion among those at higher risk for the development of TB disease like child contacts or HIV-infected individuals. This review could not find out studies which can demonstrate that interventions improved treatment completion.

The systematic review of research on implementation of interventions in TB control in LMIC is comparable to the present review.[24] This manuscript analysed the interventions from the five WHO recommendations of interventions for TB control. The review identified 73 studies among which 59 were on isoniazid preventive therapy and HIV infection. There were

substantial evidences for scale up of the five interventions at country level. And the studies available had limitation regarding design, geographic distribution and the setting of the study.

A Cochrane Review analysed the effect of tobacco smoking cessation interventions on the treatment outcomes of people with adult pulmonary TB and found a limited high-quality evidence on randomised controlled trails testing the effectiveness of smoking cessation interventions in improving TB treatment outcomes.[25] The authors concluded that short and long-term trials were needed to assess the effect of smoking cessation interventions on TB treatment.

Another study from Malaysia evaluating the additional impact of smoking cessation intervention to conventional DOTS for TB control was reported specifically for LMICs.[26] The study was conducted among 120 samples who were smokers at the time of TB diagnosis. The main intervention given was smoking cessation intervention with DOTS. The TB cure and treatment success rate was 97.5% in the integrated intervention group, which was significantly higher than that of 78.3% in the comparison group.

4.3. Studies on implementation gaps in TB control interventions

This section discusses the major gaps in implementation of intervention for TB control. There were 13studies finally selected in this category. The studies are from different LMICs including India, Israel, South Africa, Ethiopia, Bolivia, Ecuador and Paraguay, China, Greenland, Afghanistan, Africa, Gharbia Governorate and Pakistan.

In a study from Egypt reported the findings of the evaluation of the Primary Health Care (PHC) service performance in National Tuberculosis Control Program in Gharbia Governorate.[27] The major gap identified in this study was that PHC physician's lack proper knowledge about TB and their units lack proper equipment. So, there was a need for training and equipment for better performance of the health system in order to control TB program in the country.

In Pakistan, lack of access to TB services was a barrier for empowering TB patients.[28] Partnership between public, private and government sectors in treating TB as well as in improving the quality of the health care system is urgently needed in the country. The main gap identified was the lack of private sector involvement is the National TB Control program in Pakistan.

Using the review of program evaluation and the in-depth interview with key informants Seddiq et al studied the national TB control program in Afghanistan.[29] Along with the high commitment from the government, strong leadership of the program, effective partnership and coordination among stakeholders and adequate technical and financial support from the development partners, co-ordination and service delivery issues in remote areas, lack of trained staff, donor dependence were the sustainable issues identified in the program.

An observational descriptive cross-sectional study was reported from India to find out the gaps at the level of patients, or health providers in implementing RNTCP and find out the reasons.[30] In this quantitative study 106 outpatients enrolled as pulmonary tuberculosis cases in the selected medical college. The service issues identified were, first contact with unqualified local practitioner with delay in advising sputum examination in Government health facility, long distances from facility, non-availability of drugs and staffs with refusal to supply drug, inconvenient timing of clinic, fear from social stigma and fear of side effects of drugs with long duration of treatment were cited as negative factors in treatment. Other gaps identified were the significant association of socio-economic status with delay in diagnosis and initiation of treatment.

With the objective to explore barriers and facilitators to health seeking and adherence to treatment, Mabunda JT et al published a community based participatory study from South Africa.[31] Both Focus group discussions (FGGDs) and workshops were conducted. In the workshops with the planning groups, the planning people review and analyse the existing programs followed by development of a practical application. In FGDs, professional nurses, DOTS supporters, community members, and patients and the agenda for FGDs was discussed in the planning people workshops.

A qualitative study from Ethiopia aimed to investigate the experiences from both TB patients and health care providers' perspective of implementing DOT for the full course of TB treatment using in-depth interviews and focus group discussions.[32] The major implementation gap identified were: the difficulty in making a daily visit to health facility for DOT due to the distance of the facilities from their place of residences and difficult in terms of their work and social lives. Lack of transportation and high transportation cost were also found to be the main implementation gaps of DOTS in Ethiopia.

Rensburg et al in 2004 discussed social research as an intervention tool in tuberculosis control. This study was done in South Africa targeting TB patients visiting PHC and hospitalised patients, DOTS supporters and TB coordinators and health care workers.[33] Information generated by social research was used as an intervention. The findings of the study indicated that the mode of intervention was crucial in influencing TB health policy. The role of social scientist was often ignored even though they were playing major positions and roles in the health system. This was a major gap in the implementation of TB control programs.

Another study from Ethiopia to assess the impact of the expansion of the DOTs strategy on TB case findings and treatment outcome was a quantitative study based on 136,572 patients with all forms of TB registered from 1995 to 2004 in reports of TB patients treated since the introduction of DOTS in the region.[34] The program achieved 85% treatment success and 45% case detection rate from 53% to 22% respectively. The need to increase the case detection rate to WHO recommended level of 70% was the main gap identified in this study. Increase in the number of diagnostic and treatment centres did not yield to proportional increase in the number of detected cases. This was mainly due to socio-economic obstacles even though the increase in such centres improves physical access to health care.

A qualitative study to describe the methods, results, and lessons of rapid expansion of DOTS in India was published by Khatri and Frieden in 2002.[35] The main finding indicated that appropriately designed and effectively managed DOTS

programmes can achieve high case-detection and cure rates even with suboptimal technology and a suboptimal public health infrastructure. The first challenge identified was the difficulty in expanding the program in other parts of the country where it is already not covered mainly due to comparatively poor health infrastructure facilities. The second challenge was to increase the reach of the programme in areas where it was already functioning by ensuring that a greater proportion of patients was treated. The third challenge was to ensure the sustainability of the programme. The fourth challenge was to establish patient-centred services, one of the essential ingredients of successful DOTS implementation. The fifth challenge was to confront MDR-TB, which was present in 1–3.3% of new patients in the districts that have been surveyed. The sixth and most serious challenge was that presented by the epidemic of human immunodeficiency virus (HIV) infection.

An article by Waisbord assessed the challenges in reducing TB in prisons in Bolivia, Ecuador, and Paraguay and propose ways to address them through communication interventions.[36] The main challenges identified were stigmatizing attitudes and low knowledge about TB among inmates and key prison personnel discouraged people living in prisons from seeking diagnosis and treatment. Systemic problems in prison health services, along with squalid living conditions, lack of coordination between national TB programs and prison health systems, and insufficient allocation of resources to health prevented the provision of adequate TB prevention and care.

Two systematic reviews were identified. One was the summary data on the implementation of the DOTS strategy in China in terms of actual observation and treatment adherence, and to review the effectiveness of quality improvement interventions for tuberculosis (TB) control in Chin.[37] The main interventions identified were patient health education, regular follow-up, personal training and improved drug management, establishment of personal records, electronic information management, communication using the internet, use of telephone reminders and various management and transport subsidies and psychological counselling and fee for DOT. The findings of the review indicated that treatment effects of the different types of care providers and quality improvement interventions did not differ significantly. The results of the meta-analysis suggest that community involvement was effective in terms of patient outcome. The main gap in implementation identified was the need for community level participation in TB control since the DOTS program in China mainly focussed on setting up drug distribution points. Inadequate financial incentive offered to DOT observers was another key barrier identified for the implementation of DOTS.

Another systematic review based on 75 studies, 18,294 patients across 31 countries identified strategies for reducing treatment default.[38] Lower default through the use of community health workers, standardised regimens/systems and combination of adherence interventions provide effective for reducing TB. The need for comparative prospective studies to strengthen the evidence base to allow better decision making in practice and policy where resources are limited to achieve the goal of universal access to effective MDR-TB treatment was identified as the major challenge in published work.

5. Discussions

Even though almost all interventions manuscripts we got presented positive effect of intervention on TB control, generally we found limited evidences to demonstrate the best interventions for the control of TB in low and middle-income countries. Therefore, the present study failed to prioritize the ideal type of intervention applicable to LMICs. However, based on the available findings, effective interventions were more or less regional specific and not generalizable.

The three RCTs implemented three types of intervention; socio-economic, psychological and educational interventions to the patients as well as the TB supporting staff. There was no common component in these three studies. However, the important point from the findings of the RCTs in this review was the positive outcome of interventions on TB control and management. In India, most of the TB intervention research concentrated on studies to assess the role of national program on TB control. The National TB control program in the country was the most widespread and effective one in Indian situation. The community-based studies on interventions in TB control carefully indicated the area-based needs of different interventions for control of TB. So, the implications of the study findings are applicable to similar setting and cannot be generalizable.

The findings from Haiti indicated better outcome of TB control by delivering treatment in patient's homes with direct observation by former TB patients even in areas with poverty and HIV infection. Role of smoking cessation on TB control was established based on some individual study findings. Even though isoniazid preventive therapy is a main category of health intervention on TB control, we could not find a clear evidence on the effect of such intervention on TB control outcome. Findings from South Africa, which is an examples of high TB burden country doing relatively well in terms of some of the indicators associated with TB incidence, showed relative role of community-based interventions in TB control. Implementation of some joint interventions were seen only in a few studies. Our search showed that some of the studies were pilot studies and long term follow up studies were limited.

Most of the review-based studies focussed on LMICs. Role of informal community movements and other participatory approach was found to be important for the success of TB control programs. Among the different type of the interventions, DOTS was found to be the most effective program for control of TB. We found a weak association for the role of isoniazid preventive therapy (IPT) on TB control.

According to WHO, more than 20%of the global TB burden was attributable to smoking tobacco. However, TB control strategies have paid relatively less attention to smoking cessation activities as a means of TB control strategy. It was also interesting to note that countries with high level of smoking also had high burden of TB. Smoking cessation was not addressed well as an intervention to control TB. This might happen at the stage of title screening since most of the study connecting smoking and TB did not include intervention as a usage in their study titles.

Health system strengthening was a main area identified as a gap in the implementation of TB control programs. Capacity

of various level health staff were not utilized properly in most of the health systems in LMICs. Lack of accessibility in utilizing health facilities for TB treatment was another major gap identified in most of the LMICs. Insufficient community involvement was identified as the main gap in the effective implementation of TB control programs in most of the countries. Study findings from different countries indicated the heterogeneity of different interventions and the applicability of it in each geographic area. In case of India as indicated,[39] each state has its own priority and applicability for different TB control interventions.

6. Limitations

One of the major limitations of this review was our inability to identify studies which help to prioritise the best practising interventions in TB control in LMICs. Since we have included all study designs, neither could a pooled analysis nor a meta-analysis be conducted due to heterogeneity, nor could summary measures like effect size be calculated. Apart from our efforts to include relevant studies in this systematic review, there is a chance for missing of some related studies.

Heterogeneity of studies in this review makes conclusion difficult and thus the findings of this review are tentative and propose the need for further research in this area. Another limitation of this study was that we have included full text manuscripts published in English. Finally, this study is a subjective summarization of the literature on the specific subject under consideration rather than a scientific outcome.

7. Conclusion

This systematic review provides evidences over a period of more than 15 years on intervention and implementation gaps in interventions for TB control programs in LMICs. Most of the studies on implementation of TB control program supported the encouraging effect of intervention in the control of TB. The specific need of each country is clearly reflected in many of the selected studies. Even though smoking cessation was considered to be a key element of TB control, we could find limited number of published intervention trials in this area which was a major gap found in this study. To address the association of intervention and its implementation gaps, more rigorous evaluation methods are needed including meta-analysis.

Declarations

Ethics approval and consent to participate

Formal ethical approval is not required as primary data will not be collected.

Consent for publication

All authors agreed to read the final manuscript and agreed to participate.

Availability of data and material

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

The authors have none to declare

Authors' contributions

AIK, MGK conceived the idea, planned and designed the study protocol. MGK designed the figure and wrote the first draft; ALR planned and done the data extraction. MGK and AIK analysed and interpreted the studies. All authors have approved and contributed to the final written manuscript.

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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.2019.04.006.

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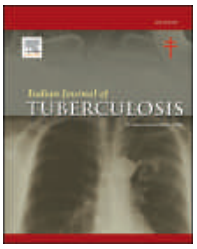

Review article

Second-line injectable induced ototoxicity in drug resistant tuberculosis: A systematic review of Indian studies

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ABSTRACT

Second-line injectables (SLIs) form an essential class of agents in the treatment of drug resistant (DR) tuberculosis (TB). However, their use is sometimes limited due to serious adverse events like ototoxicity and hearing loss, leading to permanent hearing loss if SLIs are continued. Globally as well as in India a wide variation in incidence of ototoxicity/hearing loss has been reported in patients with DR-TB. In this systematic analysis, we attempt to ascertain the ototoxicity of SLIs in Indian patients with multidrug resistant tuberculosis (MDR-TB) wherein ototoxicity onset was assessed using audiometry performed at both pre- and post-SLI treatment initiation. Twenty two studies were identified based on the inclusion criteria. Ototoxicity was observed in 10.12% [349/3447] patients within 3.8 ± 2.6 months of treatment initiation when the ototoxicity was assessed either with or without audiometry assessment. Only five studies reported ototoxicity assessment with PTA at both pre- and post-SLI initiation and ototoxicity was observed in 27.01% (121/448) patients in these five studies. Sensorineural loss was observed in three studies (high frequency loss: capreomycin, 25.0% [1/4 patients]; amikacin, 19.7% [12/61]; kanamycin, 13.3% [22/166]; streptomycin, 11.8% [2/17]; flat loss: amikacin, 8.2% [5/61]; streptomycin, 5.9% [1/17]; kanamycin 4.8% [8/166]). Most of the patients experiencing ototoxicity were managed by discontinuing (49.6% [120/242]) or replacing SLI treatment (40.8% [49/120]). The study identified high prevalence of ototoxicity in Indian patients with DR-TB treated with SLI when ototoxicity was monitored regularly using PTA (27.01%), warranting a need to

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develop unified guidelines for monitoring ototoxicity, improving physician awareness and educating patients/caregivers for reporting symptoms of hearing loss.

1. Introduction

Drug-resistant (DR) tuberculosis (TB) is a growing concern both in developing and developed countries. Multidrug resistant tuberculosis (MDR-TB), defined as a simultaneous resistance to both isoniazid and rifampicin is the most common form of DR-TB.[1] In India, an estimated burden of TB and MDR/RRTB is ~2.8 million TB cases and 1, 47,000 cases respectively in 2016. MDR-TB incidence is around 1/4th of the entire world incidence.[2,3] Second-line injectables (SLIs) treatment is an essential part of management of DR-TB. As such, the world health organization (WHO) recommends a five-drug treatment regimen (one fluoroquinolone, one SLI, two second line agents and pyrazinamide) for a period of nine to 27 months of which the SLI treatment ranges from four to nine months depending on the WHO regimen opted.[1] While treatment adherence is key to the patient outcomes, the adverse drug reactions associated with second line anti-TB drugs impact the patient adherence to the treatment.[4]

The SLIs though an important class of agents in the treatment of DR-TB, their use is sometimes limited by adverse reactions including nephrotoxicity, ototoxicity (hearing loss) and vestibular toxicity (vertigo, ataxia, dizziness).[1] SLI-induced ototoxicity initially affects high frequency, then gradually extend to low frequency sounds and ultimately results in complete hearing loss.[5] The high frequency hearing loss will not be noticed by the patients and if these are not detected at an earlier stage, continued use of SLIs may lead to permanent deafness.[6] The SLIs enter the outer hair cells and destroy them either through the disarray of stereocilia leading to apoptotic cell death or interacting with transition metal ions to produce reactive oxygen species, which damage the cells through an oxidative process,[5] blocking the transmission of sound and balance signals carried through the cochlear and vestibulocochlear nerve, respectively to the central nervous system (CNS).[7] The incidence of ototoxicity varies with the duration and dose of the SLIs resulting in high incidences of ototoxicity and hearing loss.[8–12] The programmatic management of DR-TB (PMDT) guidelines recommend evaluation of hearing loss at baseline and at every clinic visit to prevent permanent loss in hearing.[3] However, in practice most of the studies in Indian patients, determine ototoxicity based on symptoms and few studies have measured it on a regular basis irrespective of the symptoms, thus missing early ototoxicity detection. Moreover, there is a wide variations (3–100%) in the reported indices of ototoxicity.[6,13] In the absence of any well-designed Indian studies, in the present systematic review, we attempt to ascertain the ototoxicity of SLIs in patients with DR-TB. Furthermore, the article discusses the relevance of the findings in context with the published WHO and Indian guidelines.[3,14]

2. Methods

For this systematic review, standard WHO classification of DR-TB was used, which included mono-resistant (resistance to only one first-line anti-TB drug), poly-resistant (resistance to more than one first-line anti-TB drug except both isoniazid and rifampicin), multi-drug resistant (MDR-TB; resistant to both isoniazid and rifampicin), extensively drug resistant (XDR-TB; resistant to both isoniazid and rifampicin, any one fluoroquinolones and at least one of the second-line injectable agents [amikacin, capreomycin or kanamycin]) and rifampicin resistant (RR-TB; resistance to rifampicin detected using phenotypic/genotypic methods, with/without resistance to other anti-TB drugs and includes any resistance to rifampicin in the form of mono-/poly-/MDR-/XDR-resistance).[15]

2.1. Search strategy

The database of National Library of Medicine, PubMed, was used to search the articles on DR-TB in Indian population. The Medical Subject Headings (MeSH) keywords used to retrieve the articles were *hearing loss, auditory, ototoxic, cochlea, vestibular, tuberculosis, MDR-TB, multidrug resistant tuberculosis, drug resistant tuberculosis, DR TB, rifampicin resistant tuberculosis, poly-resistant tuberculosis, aminoglycosides, kanamycin, amikacin and capreomycin*. Boolean operators "OR" and "AND" were used with the keywords to make the search more specific. The keyword, "India" was not used in the search as it was possible that some of studies conducted on the Indian population may not necessarily contain the word, "India" in the title. In addition, articles from the Indian database, IndMed (http://indmed.nic.in/indmed.html) were retrieved using similar search terms. Database of World health Organization and Central TB Division India were also searched. Furthermore, cross references from individual articles were searched for any additional studies.

2.2. Search string

The search string was as follows: (hearing loss OR auditory OR ototoxic OR cochlea OR vestibular) AND (tuberculosis OR mdr tb OR multidrug resistant tuberculosis OR drug resistant tuberculosis OR dr tb OR rifampicin resistant tuberculosis OR rr tb OR poly resistant tuberculosis) AND (aminoglycosides OR kanamycin OR amikacin OR capreomycin OR streptomycin)

2.3. Data extraction

Two reviewers (DTS, PN) screened abstracts for relevance and both independently reviewed each eligible full text article using the inclusion and exclusion criteria (given below).

Conflicts over inclusion were resolved with an in-person discussion and with a third investigator (VS), wherever necessary. Once included, the data was extracted from each article and tabulated in an excel spreadsheet under different categories such as: name of the author, year, nature of study (prospective/retrospective), region, method of diagnosis, treatment, audiometry, sample size, mean age, sex and number of patients with ototoxicity.

2.4. Quality of included studies

The quality of the study was evaluated by two authors (DTS and PN) to assess the methodological quality of the included studies, including the risk of bias in the selection of the study groups (i.e., people diagnosed with DR-TB) and outcome ascertainment (i.e., hearing loss/ototoxicity).

2.5. Inclusion/exclusion criteria

The studies (1) conducted in DR-TB patients exclusively in India, 2) using SLI, 3) experiencing AEs such as hearing loss, auditory, ototoxic, cochlea, vestibular) and 4) published in English language were included irrespective of whether these report the use of specific diagnostic test for identifying ototoxicity such as the pure tone audiometry (PTA) or not. This was due to the practical limitations associated with some studies, as such rural settings where diagnostic facilities may be limited or inability of the patients to report to higher centers equipped with the facilities for hearing assessment. Short communications, consensus guidelines, and reviews were excluded from the present paper.

2.6. Statistical analysis

The data was presented descriptively and the ototoxicity estimates were compared by sex, and the type of SLIs. The pooled mean was calculated as per the standard Cochrane formula described for systematic reviews i.e., (N1*M1+N2*M2+N3*M3)/(N1+N2+N3), where N is the number of observations for respective groups; M is the mean of respective groups.

3. Results

3.1. Summary of literature search

The total number of articles identified through the literature search were 277 (PubMed $n = 217$, IndMed $n = 49$, cross references $n = 10$, google search, $n = 1$). Out of these, a total of 22 articles were included in the present systematic review based on the inclusion criteria (Fig. 1). Only seven of the total 22 articles were indexed (IndMed, n = 5; PubMed, n = 2).

3.2. Key study characteristics

The summary of characteristics of all the articles are given in Table 1. The total number of patients affected with DR-TB from all the included studies was 3447 (N) and the pooled mean from the studies reporting mean age was 33.50 years. The number of men affected were higher (2208/3447 [64.1%]) compared with the women. Most of the studies (19/22 [86.4%]) comprised patients administered RNTCP recommended standardized treatment regimen for DR-TB while the comparison between the effect of different types of SLIs (kanamycin, amikacin and capreomycin) or streptomycin on ototoxicity was carried out in two studies.[4,16]

3.3. Ototoxicity and second-line injectables

3.3.1. Ototoxicity assessment

Of the total 22 articles, ototoxicity was assessed with PTA in nine (40.9%) studies, out of which seven were prospective and two were retrospective studies (Table 1). Six of these studies conducted pre- and post-treatment PTA to assess the hearing loss of which three studies also conducted either weekly or monthly PTA assessments. The remaining three studies conducted PTA to ascertain only post-treatment ototoxicity.

Fig. 1 – Flow diagram for the selection of studies.

Table 1 – Summary of study characteristics.

Author, year, (reference)	Journal indexing[a]	Study	Region	SLI	PTA	Sample size	Men	Mean age (years)	Ototoxicity n (%)	Outcome reported	Post-toxicity SLI
Bharadwaj et al, 2015,[43]	No	P	Chhattisgarh	K	Yes	150	108	34.8	33 (22.0)	HL	DC
Dhingra et al, 2008,[44]	Yes	P	Delhi	K	NS	27	19	36.4	4 (15.0)	HL, VT[e]	NS
Duggal et al[b], 2007,[4]	No	P	Himachal Pradesh	A, K, C	Yes[c]	64	39	39.9	12 (18.7)	HL	DCC (PAS)
Nizammudin[b] et al, 2015,[16]	No	P	Uttar Pradesh	A, K, S	Yes[c]	84	55	39.9	19 (22.6)	HL	CNTD
Prasad et al, 2006,[45]	No	P	Uttar Pradesh	K	NS	46	26	30.2	4 (8.7)	HL, VT[e]	DC
Prasad et al, 2016,[25]	Yes	P	Uttar Pradesh	K	NS	98	68	29.3	28 (28.6)	HL, VT[d]	DCC
Sharma et al[$], 2016,[17]	No	P	Punjab	K	Yes[c]	100	68	37.5	18 (18.0)	HL	DCC
Singh et al, 2007,[46]	Yes	P	Maharashtra	A, K, C	Yes	56	42	30.3	6 (10.7)	HL	DC
Tiwari et al, 2016,[19]	No	P	Gujarat	K	Yes[c]	100	61	16-45 age (89%)	37 (37.0)	HL	DC
Zala et al, 2015,[18]	No	P	Gujarat	K	Yes[c]	100	61	28.8	35 (35.0)	HL	NS
Bhushan et al, 2014,[47]	Yes	P	Punjab	K	NS	207	131	12 to 68	27 (13.0)	HL, VT[d]	DC; DM
Joseph et al, 2011,[48]	Yes	P	Tamil Nadu	K	NS	38	25	<30 = 11; 30–45 = 11; >45 = 16	15 (39.5)	HL, VT[d]	DC; DM
Akshata et al, 2015,[13]	No	R	Karnataka	K	Yes	607	402	NS	18 (3.0)	HL, VT[e]	NS
Akshata et al, 2016,[36]	No	R	Karnataka	K	NS	69	46	35.8	5 (7.2)	HL, VT[e]	DM
Arora et al, 2007,[49]	Yes	R	Delhi	K	NS	66	36	28.0	3 (4.5)	HL, VT[e]	DC
Dela et al, 2017,[50]	No	R	Gujarat	K	NS	125	89	35.7	6 (4.8)	HL	DC
Kapadia and Tripathi, 2013,[51]	Yes	R	Gujarat	K	NS	63	40	34.0	3 (4.8)	HL, VT[e]	DCC
Nair et al, 2017,[52]	No	R	Kerala, Delhi and West Bengal	K	NS	788	535	most patients aged 15-44	34 (4.3)	HL	NS
Patil et al, 2017,[20]	No	R	Maharashtra	K	NS	468	287	34.5	21 (4.5)	HL, VT[d]	DCC (PAS)
Singla et al, 2009,[53]	No	R	Delhi	K	NS	126	68	26.0	5 (4.0)	HL, VT[e]	DC
Sood et al, 2016,[54]	No	R	Himachal Pradesh	K	NS	62	NS	40.79	13 (21.0)	HL, VT[d]	NS
Dhananjay and Shabnam, 2017,[6]	No	R	Uttarakhand	K	Yes[c]	3	2	27.6	3 (100.0)	HL	NS

[a] Indexed, PubMed or IndMed.
[b] complete otolaryngologic examination conducted.
[c] pre-and post-treatment.
[d] studies reporting both hearing loss and vestibular toxicity separately.
[e] studies reporting combined incidence of HL and VT; A, amikacin; C, capreomycin; CNTD, continued; DC, discontinued; DCC, discontinued and SLI changed; DM, dose modified; HL, hearing loss; K, kanamycin; NS, not specified; P, prospective; PAS, para-aminosalicylic acid; PTA, pure tone audiometry; R, retrospective; S, streptomycin; SLI, second-line injectable; VT, vestibular toxicity (giddiness, tinnitus and vertigo).

Furthermore, complete otolaryngologic examination as a part of audiologic investigation was performed in 3 studies (3/9 [33.3%]).[4,16,17] Tympanometry and questionnaire-based ototoxicity assessment was used in one study[18] each.[6,18] Of the studies using PTA, three studies categorized the sensorineural loss due to ototoxicity as low frequency loss (LFL, hearing loss at frequency less than 2000 Hz), high frequency loss (HFL defined as a ≥20 dB decrease at any of the three frequencies [4000, 6000 and 8000 Hz], ≥10 dB decrease at any two adjacent frequencies in the above range and loss of response at all the three frequencies) and flat or dead ear (ear not responding to any frequency).[4,16,17] Of the 22 articles included, 10.12% (349/3447) patients reported ototoxicity assessed either with or without audiometry assessment. Only nine of these 22 studies assessed ototoxicity using PTA (prospective, n = 7; retrospective studies, n = 2) and five of these nine studies prospectively assessed ototoxicity at both pre- and post-SLI initiation. The incidence of ototoxicity was 14.32% (181/1264) when ototoxicity was assessed with PTA (i.e., 9/22 studies) and 27.01% (121/448) when the PTA assessments were performed both pre and post-treatment initiation (i.e., 5/9 studies). Thirteen studies conducted ototoxicity assessment without PTA audiometric assessments. Seven of these thirteen reported hearing loss, five reported otovestibular toxicity and one reported combined hearing loss, and vestibular toxicity. The incidence of ototoxicity was 7.70% (168/2183) in patients with audiometric evaluation without PTA. A total of 15 (15/22 [68.2%]) studies reported the incidence of hearing loss and seven (7/22 [31.8%]) studies reported cases experiencing combined hearing loss and vestibular toxicity (giddiness, tinnitus and vertigo). Of the 15 studies reporting hearing loss, five of them also mentioned the incidence of other vestibular toxicity symptoms.

Hearing loss was observed in 72% (251/349, mean 16.7 ± SD 12. 3) patients, combined hearing loss and vestibular toxicity in 12% (42/349, mean 6.0 ± 5.4) patients and exclusive vestibular toxicity in 16.0% (56/349, mean 11.2 ± 4.9) patients.

Out of the three studies estimating sensorineural loss, HFL was noted in 25% (1/4) patients with capreomycin treatment followed by 19.7% (12/61) with amikacin, 13% (22/166) with kanamycin and 11.8% (2/17) with streptomycin (Table 2). While LFL was not observed with capreomycin and streptomycin, one patient each in kanamycin (1/166) and amikacin (1/61) exhibited LFL. Flat loss or dead ear was seen in the participants receiving amikacin (8.2% [5/61]), streptomycin (5.9% [1/17]) and kanamycin (4.8% [8/166]). The use of capreomycin was not associated with flat loss or dead ear. Only one study which reported bilateral or unilateral hearing loss[17] found bilateral loss in majority of the HFL 69.2% (9/13) and all of the dead ear (4/4) cases.

A total of 15.50% (86/555) and 9.09% (263/2892) patients reported ototoxicity in articles published in indexed and non-indexed journals, respectively. The use of PTA for audiometry assessment was invariably higher for articles from non-indexed journals (53.3% [8/15]) versus those from indexed journal (14.28% [1/7]).

3.4. *Onset of ototoxicity*

The mean duration for onset of symptoms of ototoxicity (based on the studies reporting, $n = 12$) was 3.8 ± 2.6 months for the SLIs. For the individual SLIs, the symptoms for ototoxicity were noted in 2.5 (amikacin, $n = 2$), 3 (streptomycin, $n = 1$), 4.0 (kanamycin, $n = 12$) and 4.0 (capreomycin, $n = 1$) months. In the categories for sensorineural loss, earliest to occur were LFL and HFL (3 months [amikacin, kanamycin and streptomycin]) while the dead ear was noted in 6 months (amikacin and streptomycin).[16]

3.5. *Management of ototoxicity*

Ototoxicity management with discontinuation or replacement of the drug was reported in 15 (15/22 [68.2%]) studies comprising 242/349 [69.4%] patients. Discontinuation is the most common method used to manage ototoxicity and was noted in 49.6% (120/242) patients while dose reduction was observed in 5.4% (13/242) patients.[4,17,19] The replacement of discontinued SLI was reported in 40.8% (49/120) patients but only two studies (2/5) reported the replacement drug, para-amino salicylic acid (PAS)[4] (Table 3).

4. Discussion

The present systematic review attempted to analyze the prevalence of ototoxicity from India and to determine whether ototoxicity can influence decision making while managing patients with DR-TB using SLIs. The varying incidences of ototoxicity (3%–100%) with SLIs identified in this systematic analysis[6,13] is in concordance to the globally reported variations in incidence of ototoxicity and hearing loss (4%–62%).[8–12] However, variability exist between global and Indian studies, with Indian studies reporting higher variations, probably due to

Table 2 – Summary of ototoxicity due to SLIs in drug resistant tuberculosis.

Aminoglycoside	Number of studies	n	Sensorineural loss (n=248)			Onset of ototoxicity (months)
			n (%)			
			HFL	LFL	Dead ear/Flat loss	
Amikacin[4,16]	2	61	12 (19.7)	1 (1.6)	5 (8.2)	2.5
Capreomycin[4]	1	4	1 (25)	–	–	4
Kanamycin[4,16,17]	3	166	22 (13.3)	1 (0.6)	8 (4.8)	2.8
Streptomycin[16]	1	17	2 (11.8)	–	1 (5.9)	3

HFL, high frequency loss; LFL, low frequency loss, N: total number of patients; n, subgroup of total patients; SLI, second-line injectables.

Table 3 – Influence of ototoxicity on treatment outcome.

Study	Sample	Discontinuation	Change	Changed drug	Dose modification
Bharadwaj et al, 2015,[43]	33	5	–	–	–
Dhingra et al, 2008,[44]	4	–	–	–	–
Duggal et al, 2007,[4]	12	12	12	PAS	–
Nizammudin et al, 2015,[16]	19	Not stopped	–	–	–
Prasad et al, 2006,[45]	4	2	–	–	–
Prasad et al, 2016,[25]	28	3	2	–	–
Sharma et al, 2016,[17]	18	18	18	–	–
Singh et al, 2007,[46]	6	6	–	–	–
Tiwari et al, 2016,[19]	37	37	–	–	–
Zala et al, 2015,[18]	35	–	–	–	–
Bhushan et al, 2014,[47]	27	6	–	–	4
Joseph et al, 2011,[48]	15	1	–	–	5
Akshata et al, 2015,[13]	18	–	–	–	–
Akshata et al, 2016,[36]	5	–	–	–	4
Arora et al, 2007,[49]	3	3	–	–	–
Dela et al, 2017,[50]	6	5	–	–	–
Kapadia and Tripathi, 2013,[51]	3	3	3	–	–
Nair et al, 2017,[52]	34	–	–	–	–
Patil et al, 2017,[20]	21	14	14	PAS	–
Singla et al, 2009,[53]	5	5	–	–	–
Sood et al, 2016,[54]	13	–	–	–	–
Dhananjay and Shabnam, 2017,[6]	3	–	–	–	–

Abbreviations: PAS, para-amino salicylic acid.

limited resources to conduct audiometry or lack of specific guidelines to monitor ototoxicity.[16,17,20]

In the current systematic analysis, the incidence of ototoxicity was observed in 10.12% patients from twenty two studies when ototoxicity was assessed either with or without audiometry assessment. The incidence of ototoxicity increased to 27.01% (121/448) when ototoxicity was assessed using pure tone audiometry both pre- and post-SLI initiation. Few studies reported specifically on hearing loss, others estimated the patients with combined ototoxicity and vestibular component. The vestibular component was diagnosed based on patient symptoms, hearing loss was reported either by the patients or determined using PTA audiometry. Limited facilities available with the treatment centers in rural settings or inability of the patients from distant neighborhoods to report to the higher centers for regular monitoring could be the possible reasons for the failure to conduct audiometry in all the studies.[16,17,20] Guidelines recommend a baseline audiogram and further screening in patients with changes in audiogram,[21,22] however, this is not uniformly adhered across centers[23] and also there is a lack of unified guidelines suggesting methods of optimal management of ototoxicity in these patients. Importantly, DR-TB patients usually have a prior exposure to streptomycin and therefore, hearing should be tested before starting SLIs.[7] Additionally, programmatic management of DR-TB (PMDT) guidelines recommend baseline audiometry testing for all the patients with DR-TB and regular evaluation of the ability for normal conversation.[3] The British Society of Audiology (BSA) and the American Speech-Language-Hearing Association (ASHA) guidelines suggest to conduct testing for hearing loss at baseline, weekly or biweekly and a post treatment follow-up after few months of SLI discontinuation.[21,22] Further, it is possible that drugs with ototoxicity potential (loop diuretics, antimalarial [quinine], macrolide antibiotics, platinum-based anticancer drugs, and salicylate analgesics) when co-administered with SLIs in MDR-TB[24] may have an additive effect on SLI induced ototoxicity warranting additional investigation or controlled prescription.[25] In addition, aminoglycosides have dose-dependent ototoxicity and fluctuations in plasma levels resulting, from the route of administration, e.g., kanamycin and capreomycin are available as both iv and im injections (unlike amikacin, only iv injection) and this can affect the narrow therapeutic index and result in ototoxicity.[26] Otoscopy otoacoustic emission (OME) and tympanometry could be used as additional diagnostic aids with OME being reported as more sensitive tool to diagnose outer hair cell damage inflicted by the SLIs.[6,7]

In the current analysis, the number of men experiencing ototoxicity were relatively higher (1.8 times) versus women patients. The findings are in concordance with the earlier reported trends of relatively higher incidence of ototoxicity in men versus women and could be due to relatively lower rate of aminoglycoside elimination and hence an apparently longer half-life of aminoglycosides in men versus women.[27] No conclusive evidence based on individual SLI data on relative ototoxicity's of SLIs could be drawn in our review. However, studies have shown that all SLIs can affect both cochlear and vestibular toxicity, though some SLIs may have preferential ototoxicity e.g., lesser ototoxicity/hearing loss with capreomycin and kanamycin versus amikacin, predominantly vestibular toxicity with streptomycin.[12,28,29]

Globally several studies report ototoxicity in patients with MDR-TB however, studies using PTA or other recommended audiometric methods remain limited. A cross-sectional study from Portugal, where PTA audiometry was used to determine ototoxicity at average audiometry thresholds (500–4000 Hz) reported an incidence of 65% hearing loss with amikacin.[30] Another study from United Kingdom wherein PTA

audiometry was performed at baseline and monthly thereafter observed ototoxicity in 55% patients with amikacin and capreomycin and 40% SLI-related discontinuations.[12] Another study in Afghani/Iranian nationals with PTA audiometry performed both pre- and post-SLI treatment reported hearing loss in 70% patients on amikacin.[31]

Moreover, in the process of ototoxicity, HFL occurs first and therefore, patients themselves may not be aware of the pathology to report incidence of ototoxicity/hearing loss to the clinician.[4,16,17] Furthermore, since higher frequencies are involved before the lower frequencies, frequent monitoring may assist to detect the hearing loss early and minimize the irreversible hearing deficits.[4,13,16] SLIs are known to affect hearing exclusively or initially in the higher frequency range (9–20 kHz) before presenting to lower frequency ranges (250–8000 Hz). In addition, SLIs are accumulated within the inner ear fluid and are eliminated slowly.[32] Thus studies that report hearing loss by patients at close to lower frequencies or screening at conventional frequencies (250–8000 kHz) or those with limited screening post SLIs treatment, often underreport SLI ototoxicity.[32] Thus there is an increasing need to establish operational protocols in close relationship with screening centers and ENT departments, to overcome the underreporting of ototoxicity associated with SLIs and improve early detection and prevention.[30]

Even though irreversible hearing loss is well-known with the SLIs,[15] SLIs continue to be used due to the lack of equally effective alternative and as an economically viable option in developing nations.[7,17,33] Administration of otoprotective agents such as NMDA receptor antagonists (Ifenprodil), compounds with known antioxidant capacity such as N-Acetylcysteine, Methionine (D-Met) and α-lipoic acid (α-LA), vitamins such as α-tocopherol (vitamin E) and vitamin C as well as the herbal extracts Gingko biloba, in combination with or before SLIs have been explored for protection and treatment of SLI ototoxicity.[34,35] The PMDT India guidelines advise reducing the dose of SLI to twice/thrice a week or replacement with capreomycin. However, with continued hearing loss, the guideline recommends SLI discontinuation in consultation with the patient.[3] In most of the current studies (15/22 [68.2%]) reviewed, on finding hearing loss, SLIs were either dose adjusted, discontinued or replaced with drugs like PAS, which may have resulted in preventing the further incidence of hearing loss.[4,13,16,36,37] Other factors that can be considered for managing ototoxicity due to SLI is therapeutic dose monitoring, however, very limited data has been reported on SLI bioavailability especially in patients with DR-TB.[7] In countries where aminoglycosides are used widely, a quarter of the people with hearing loss induced by aminoglycosides have maternal relatives with deafness related to drug-induced ototoxicity. This suggests presence of an inherited predisposing mutation (usually m.1555A > G, a mitochondrial DNA mutation). Hearing loss induced by aminoglycosides in individuals with the m.1555A > G mutation can be prevented provided these are identified earlier. However, most of the available genetic testing methods are time consuming and delaying the treatment in MDR patients could further deteriorate their condition. Therefore, there is also a greater need for rapid genetic methods for identifying ototoxicity conducive mutations.[38]

A window of opportunity may appear early in the treatment with altered audiogram warranting modification/discontinuation of the treatment before the patients become symptomatic, however this opportunity is not always utilized.[23] Khanna et al observed 5% patients with hearing impairment two months after SLI treatment initiation based on baseline audiometry evaluation. Later based on symptomatic screening for hearing loss, SLIs were stopped/discontinued in 50.6% (43/85) patients because of ototoxicity.[39]

The drug development in MDR-TB continues to evolve and delamanid and bedaquiline have been recently added to the list. The interim policy guidelines of the WHO recommend the conditional use of delamanid and bedaquiline in the regimen of adult patients with pulmonary MDR-TB especially with drug intolerance and resistance to fluoroquinolones or injectable drugs.[14,40] In India, bedaquiline was made accessible to MDR-TB patients at six sites across the country for the conditional access programme under RNTCP in 2016.[41] The RNTCP plans to expand access to Bedaquiline® pan-India and also introduce Delamanid® after approval, and explore if the combination therapy could reduce the regimen of MDR-TB to 4–6 months.[3] Finally, it must be emphasized that the key to ototoxicity prevention and management is early diagnosis, appropriate treatment in an accessible manner, effective physician-patient communication including counselling and education of the patients, family members and caregivers.

The systematic review has several limitations and could have included patients in TB endemic countries and experiencing SLI adverse effects such as nephrotoxicity. However, as there is wide variation in reporting ototoxicity and our goal was to highlight the importance of performing highly sensitive audiometric evaluations such as using pure tone audiometry at baseline and at regular interval to minimize the toxicity and highlighting the need for early intervention (i.e., modifying DR TB treatment regimen), we limited the scope only to studies reported in Indian population and to patients experiencing ototoxicity with SLIs. Treatment interventions for management of hearing loss and therapeutic drug monitoring were not studied during this. Further, as there were limited number of studies, the systematic review included few studies which were published in low impact factor journals or non-indexed. The authors decided to include these studies in order to avoid any study selection bias.

5. Conclusion

Ototoxicity impacts the long-term patient adherence to treatment in DR-TB, which in turn is critical for the successful regimen. A high prevalence of ototoxicity was observed in Indian patients from studies which used pre- and post-treatment PTA audiometry in patients with DR-TB. Both in India and the global population variations in incidence of ototoxicity exists, necessitating baseline and periodic monitoring of ototoxicity. Furthermore, there is an unmet need to develop a unified guideline to monitor ototoxicity in patients with DR-TB on SLI treatment. It is important to increase physician awareness on monitoring ototoxicity during the treatment process and to involve them in patient counselling. Patients and caregivers need to be well-informed of the

possible adverse reactions with SLIs, report symptoms and should be involved in the decision-making process. The findings from this systematic analysis could pave a way towards early adoption of newer anti-tubercular drugs, which can be used in cases of ototoxicity with SLIs.[42]

Conflicts of interest

Dr. Deepak T.S. and Dr. Prashant Narang are employees of Janssen, Pharmaceutical Companies of Johnson & Johnson, Mumbai, Maharashtra, India. Dr. Vikram Singh is a former employee of Janssen, Pharmaceutical Companies of Johnson & Johnson, Mumbai, Maharashtra, India. Johnson & Johnson Pvt. Ltd., India is a marketing authorization holder of Bedaquiline® which is a non-injectable drug for DR-TB patients.

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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.2019.04.007.

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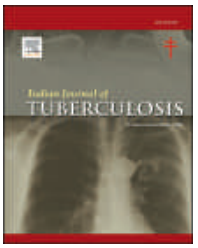

Review article

Regulating E-cigarettes in India: A conundrum for the global giant in tobacco production

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ABSTRACT

Electronic cigarettes which are termed as e-cigarettes, e-cigs or e-vaporizers are used by the people for creating the inhalable aerosol which carries nicotine in it. Also, commonly referred as vaping. E-cigarettes are used as an alternative to the regular cigarettes and aids in the cessation of smoking. However, there has been tough strife and debate regarding e-cigarettes that are accompanied in the media stories which bring different opinions among consumers, experts as well as regulators who are involved in making decisions from no regulation to regulating e-cigarettes to banning of e-cigarettes which will bring direct impact on public health. In this article, an overview about the controversy of e-cigarettes with respect to the device, its market, regulation norms of e-cigarettes at different platforms and amidst the debate over e-cigarettes banning in India has been portrayed. It is surveyed that India being a hub of around 110 million tobacco smokers and a global giant in tobacco production, where the Indian government is planning to bring a complete ban over e-cigarettes throughout the country propels an elementary question of banning safer alternatives and not regular cigarettes which makes no sense from the point of banning e-cigarettes until or unless regular cigarettes are banned. Varying point of views from experts, scientists, users with respect to e-cigarettes has been addressed which shares a mix opinion with the supporters promoting ban as well as the antagonist with the concept of regulating the e-cigarettes in India.

1. Introduction

Electronic cigarettes are battery-operated devices which are used by the mortals for creating the inhalable aerosol which accommodates nicotine in it. Electronic cigarettes are embodied with four distinct elements which cover a mouthpiece, a cartridge or reservoir, an atomizer and a power source battery.[1] The liquid is stockpiled in an atomizer which is inherent with the small heating element which generates heat

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to the resistance coil which will be encircling a wick. Generally, the liquid such as Propylene glycol, glycerol, nicotine, and flavorings are used.[2] These electronic cigarettes are termed as e-cigarettes, e-cigs or e-vaporizers which are categorized under electronic nicotine delivery system (ENDS).[1] Also, commonly called as vaping. E-cigs can be similar to the appearance of either traditional tobacco cigarettes, pipes, cigars and even usual items like USB memory sticks or pens.

A Chinese pharmacist Hon Lik was responsible for the invention of e-cigarettes in 2003. In 2007, his design was internationally patented. Around 2006, e-cigarettes gained commercial popularity in the USA and Europe.[3]

Indeed, e-cigarettes are assumed to be less virulent in contrast to the smoking as well as considering the peculiar feature of e-cigarettes which resembles smoking and procure contentment to the user so they are likely considered as most potent tobacco harm reduction products.[2]

Currently, there has been tough strife and discussions on e-cigarettes that are accompanied in the media stories which conclude disorientation among consumers, experts as well as regulators who are involved in making decisions which influences public health. There are folks as strong promoters who think that e-cigarettes aid in reduction or suspension of smoking. On another hand, there are folks as strong antagonists who assume that the e-cigarettes encourage addictive habit and threaten to the permanent approach to smoking.[2]

1.1. *E-cigarettes in the tobacco market*

By 2023, electronic cigarette market is expected to be $26, 839 million from $8610 million in 2016 globally and from 2017 to 2023 a Compound Annual Growth Rate (CAGR) of 17.4%.[4]

In the past few years, e-cigarettes have gained expanding acceptance worldwide which showed the impact on the dominant players in the tobacco industry with a seasoned downfall from the demand of the traditional cigarettes.[5]

Depending on product type, flavor, distribution channel, and region the electronic cigarettes market is segmented globally. The market is split into disposable, rechargeable and modular on the basis of product type. Flavors in e-cigs comprised of tobacco, fruit, sweet, beverage, botanical and others. Depending on distribution channels, the market is assorted into specialist e-cig shops, supermarkets, online, tobacconist, and others. Based on the region the market can be scrutinized into North America, Asia–Pacific, Europe, and LAMEA. North America is the chief revenue supplier to the international market owing to the immense perception of e-cigarettes and health endangerment due to tobacco smoking. As per the report of 2016, in US there were around 3.6% of electronic cigarettes users.[4]

In spite of the fact that the e-cigarettes shattered acceptance worldwide, India has not been so keen to enter e-cigarettes steam. India has 110 million tobacco smokers approximately. By virtue of lack of attention and comparatively high price of e-cigarette starter kits, e-cigarettes have not been endured to compete with traditional cigarettes as yet. The market is also affected by the lack of regulations. Latterly, tobacco companies accomplished the market status of e-cigarettes in India addressing to acquire a huge income where an e-cigarette figure around US$6- US$8 per stick. While the e-cigarettes market is still in infancy, there are four major vendors in cigarette market which includes ITC, Godfrey Phillips India, VST Industries and Golden Tobacco.[5]

1.2. *Differences across platforms (legal status in different countries)*

Ranging from no regulation to complete bans there are peculiar regulations of e-cigarettes across countries. The regulatory decisions are uncertain on the empirical basis and further groundwork on electronic nicotine delivery system must be carried out to assure the decisions of regulators counting safety, abuse liability and efficacy for smoking suspension.[6]

Currently, in most of the countries, e-cigarettes are treated as a consumer product and are accessible in convenience stores, specialized shops or available in the online source. Thorough ban has been imported on these products in several countries of South America and in Asia. In another plot, the regulation may be based on the nicotine presence or absence in liquid.[2]

In the USA, regulation of e-cigarettes is overseen by Food and Drug Administration were e-cigarettes are evaluated as tobacco products. FDA regulates the manufacture, import, packaging, labeling, advertising, promotion, sale as well as the distribution of e-cigarettes.

In May 2016, New tobacco rule by the FDA under Family Smoking Prevention and Tobacco Control Act was established which covered all the tobacco products, in addition to it e-cigarettes, pipe tobacco, all cigars, nicotine gels, as well as dissolvable that formerly was not classified or regulated under the authority of FDA were included in this new rule. The new rule focused on confinement to newly regulated tobacco products for youth access by following norms:

- Prohibition of sale of these products to those younger than 18.
- Age verification via photo ID to be provided on buying.
- Ban on tobacco products to be sold in vending machines.
- Products to be packed with warnings stating that they have nicotine in it.

Basically, all e-cigarettes have to pursue marketing authorization before placed on the market. The FDA has taken this action to assure Americans from tobacco-related epidemic and death.[7]

In EU, initially there was a point where e-cigarettes were meant to regulate as medicinal products in the Tobacco Product Directive but because of certain circumstances, this option was expelled by the expert groups as there was differences in their opinion and eventually was not conserved in the concluding version. The foremost reason for the dispute of such regulation was to safeguard the safety of the consumer and precise labeling of the product. From following this path resulted in unplanned outcomes.[2] As off 19 may 2014, the European Commission started regulating e-cigarettes. By 2016, Tobacco Products Directive was relevant to all the member states of European Union.[8] At present, e-cigarettes are traded as consumer products in EU as per Article 20 of the Tobacco Products Directive (2014/40/EU) which has laid following rules:

- Specifications regarding the quality as well as the safety of e-cigarettes: The maximum nicotine concentration and volume for cartridges, tanks and nicotine liquid containers have been mentioned in this directive. E-cigarettes should possess a mechanism that favor refilling without spillage in order to protect consumers. E-cigarettes should be tamper evident and resistant to the child. The ingredients used must be of high purity and deliver when puffed with the same amount of nicotine at the same strength and duration.
- Requirements for e-cigarettes with respect to packaging as well as labeling: Health warnings related to e-cigarettes should be notified to the consumers that they carry nicotine and should not be used by non-smokers are mandatory. The list of ingredients contained in the product, data on the product's nicotine content should be mentioned in the Packaging. In addition, Leaflet must be provided with the information regarding risk groups, adverse events, toxicity and addictiveness along with the instructions for use. Cross-border advertising of e-cigarettes and promotional elements on the packing of e-cigarettes is prohibited.
- Monitoring as well as developments reporting in concern with e-cigarettes: The monitoring and reporting requirements for manufacturers and importers, EU countries and the Commission are laid down by the directive.[9]

In Singapore, Health Sciences Authority (HSA) under the Tobacco (Control of Advertisements and Sale) Act prohibits the import, distribution, sale of vaporizers such as e-cigarettes, e-cigars, e-pipes and considers it as an offense for the person who contravenes the law. A fine not exceeding $10,000 or imprisonment for 6 months can be liable for those who found guilty of the offense. A fine of $20,000 or imprisonment for 12 months duration or both can be liable in case of repeat offenders.[10]

Although there is the prompt growth of industries pertaining to e-cigarettes in a country like China, e-cigarettes manufacture, sale, and its use continue to be not regulated yet. As of now, in a nutshell China's legal provisions indicate that electronic cigarettes fail to meet the rationale of pharmaceuticals, tobacco products or medical devices. Bringing standardization in e-cigarettes may require modification of existing legislation, reasonably with the help of the Standing Committee of the National People's Congress or confined to the Supreme Court judicial interpretation.[11]

In case of India, e-cigarettes regulations are not yet regulated. In the absence of requisite provisions under the Cigarettes and Other Tobacco Products Act (COTPA), the health ministry is now examining other laws such as the Drugs and Cosmetics Act and the Food Safety & Standards (Prohibition and Restriction on Sales) Regulation, 2011, to effect a ban. As of now, states such as Haryana, Punjab, Kerala, Mizoram, Karnataka, Bihar, Jammu and Kashmir has set forth a ban on e-cigarettes.[12] Out of all states, in 2013 Punjab banned e-cigarettes stating liquid nicotine as an unregistered and new form of nicotine thereby an illegal drug in India. On the other hand, due to increased addiction to the device among the youth Jammu and Kashmir has set forth ban of e-cigarettes. No clear classification is given in Drugs and Cosmetics Act, 1945 on use of nicotine with respect to e-cigarettes. Various studies have been undergoing the assurance of these devices and the fact it can easily available to children and adolescents, so some of the Indian states have taken this element into their own hands.[13] In Punjab, a man was sentenced to 3 years imprisonment and pay a fine of Rs 1 lakh for selling e-cigarettes which were very first conviction regarding e-cigarettes.[14] Table 1 discuss various pros and cons of regulatory approach pertaining to e-cigarettes.[2]

1.3. *Narration from world health Organization's framework convention on Tobacco Control (WHO FCTC)*

India was one among the 168 states that signed the WHO FCTC on February 5, 2004.[15] India has acknowledged to pursue guidelines in order to strengthen the smoking subjects to depart as well as confine non-smoker's from catching the habit.[16]

As per the report announced by the WHO Tobacco Free Initiative Commission in 2013 implied countries to regulate e-cigarettes which included prohibiting the e-cigarettes sale in the market whenever one cannot lawfully purchase cigarettes or else in locality where buying of traditional cigarettes is forbidden, prohibiting a system of cobranding of cigarettes in contrast with e-cigarette products, retailing in such a way which push for binary purpose, prohibiting the distinguishing flavors which are adopted in e-cigarettes especially flavors

Table 1 – Different Pros and Cons of Regulatory approach pertaining to e-cigarettes.[2]

Regulatory pathway	Pros	Cons
Importing Sales Ban of e-cigarettes	Helps non-intended public to avoid the utilization of e-cigarettes.	Limits the smokers from utilizing alternative product which is less adverse
Nicotine accommodating products prohibition	Helps non-intended public to avoid the intake of nicotine	Risk of Utilization of non -nicotine liquids are not focused; cessation of smoking will be intensely difficult if the nicotine is prohibited.
Regulation pertaining to Medicinal Products	Maximal safety will be ensured	Expensive; will slow down the innovation
Regulation Pertaining to Tobacco Products	Identical restrictions as that of the use of tobacco products are applicable	Provides wrong idea stating equal risk with respect to both e-cigarettes and tobacco products
Regulation pertaining to Consumer Products	E-cigarettes are utilized as consumer products	Does not focus on particular matters such as nicotine content; constitute an impact that they could be utilized by the entire population as a new practice.

such as alcohol and candy, companies are banned which creates false belief concerning cessation of tobacco or their products certified by suitable regulatory authority which commands the standards in order to bring improvement by regulating the ingredients of products as well as its functioning.[3]

1.4. *Tobacco Control legislation in India*

The Cigarettes and Other Products Act, 2003 which covers Prohibition of Advertisement and Regulation of Trade and Commerce, Supply and Distribution (COPTA) is a key law followed in India for Tobacco Control. This Act was enacted before India had become a party in WHO FCTC.[16] In 2004, the Ministry of Health and Family Welfare excised powers in Section 31 of COPTA notifying the first set of rules regarding:

- Smoke-Free Places: In many of the public places and workplaces such as healthcare, educational, public transport smoking is prohibited. Despite, the law allows the creation of smoking space in hotels owning 30 or more rooms and restaurants having seating scope for 30 or more.
- Advertising, Promotion and Sponsorship of Tobacco: Advertising through most forms of broadcast is prohibited. Limitations are imposed on publicity or attention for tobacco sponsorship.
- Packaging and labeling of Tobacco: Particulars such as "light" and "low-tar" and other signs are contraband which gives false information. As of April 2016, 85-percent pictorial health warnings on either side of tobacco packs are enforced.[17]

1.5. *A view on banning of e-cigarettes*

In India banning of e-cigarettes is a highly controversial element even in 2018. There are numerous debates on e-cigarettes and its regulations. The opinion of folks is divided finding no perfect solution. The Center under Ministry of Health and Family Welfare has a firm opinion on prohibiting of e-cigarettes which seems to be challenging task as there are supporters as well as opposers expressing their thoughts on banning e-cigarettes respectively. Fig. 1 portrait a view on the current scenario of e-cigarettes in India.[18]

As per one of the surveys over e-cigarettes, Dr. Pankaj Chaturvedi, Professor in Tata Memorial Hospital in Parel have stated that e-cigarettes are more threatening when compared to smoking cigarettes and should be banned in India as they contain propylene glycol which is considered as a prime solvent in e-cigarettes which is an industrial poison. Further, they aid in sustaining the practice than quitting it.

Fig. 1 – A portrait of E-cigarettes scenario in India.[18]

According to the director Dr. PC Gupta, HEALIS Sekhsaria Institute for Public Health, Mumbai, the flavoring agents which are used in the e-cigarettes are one more method to draw the attention to young customers. In addition, he also stated that it is just another plan of marketing a new product in the market and simultaneously supporting the old tobacco product by the tobacco industry.[19]

As per Tobacco Institute of India (TII), the ban of e-cigarettes may result in smuggling of these products and further with no guarantee on quality standards. The dominant e-cigarettes manufacturers like ITC, VST and Godfrey Phillips stated that in India, the ban of e-cigarettes will be a massive loss when compared to the countries which have adopted regulatory concept on these products. On the other side, the prime objective of the tobacco industry towards e-cigarettes is to endure profit which is drastically affected by the prohibition of e-cigarettes.[20]

Although there is an acknowledgment from Ministry of Health and Family Welfare regarding the ban of e-cigarettes, vapers are approaching jointly in public to resist such a change. E-cigarettes users who are referred as vapers are annoyed with the ban thinking that it will drive them to the routine of smoking cigarettes which are treated to be more detrimental.

A 27-year-old businessman in Delhi stated that the ban regarding e-cigarettes will push users back to cigarettes and describes the feeling of being scary as he had been smoking cigarettes continuously for a term of 5 years and had given up this habit in preference to e-cigarettes.

As per Arun Kumar Jha who is the economic advisor of Health Ministry and also involved in the control of Tobacco, stated that there is no proof that e-cigarettes reduce smoking. Also, he expressed that how the health ministry is uncertain in controlling the sale as well as the distribution of e-cigarettes because of the absence of regulatory framework and further the e-cigarettes do not fall under any legal law.

In Karnataka State, a union of 300 people in federation with vapers was formed in order to respond against the e-cigarettes bans which have been transpired but not yet correctly imposed. This union filed a litigation with respect to public concern on the sale as well as the distribution of e-cigarettes to Karnataka High Court. The final hearing by the court was given with respect to banning to e-cigarettes.

Many users of e-cigarettes say that devices have helped them quit tobacco and they conclude as e-cigarettes are better than traditional cigarettes. Users think that compared to smoking, e-cigarettes are devoid to harm. Further, vapers have set forth the idea of 'Don't Ban, Regulate' suggesting to implement regulations, rules, guidelines for the manufacture, sale as well as the use of e-cigarettes rather banning e-cigarettes in India.[21]

Some of the experts express that in India banning of e-cigs may lead to more harm where the Indian Scientists are considering banning of e-cigarettes as an epic fault and they have been claimed to legal authorities to oversee a centric

study before stepping into an abrupt move. As per one of the study, it was found that availability of e-cigarettes in India reduced the smoking number near to 50% which is equal to saving 90 million lives. Scientists namely Dr. Siddiqi and Prof. Sharan have written a letter addressing to Union Minister about the higher risk to the public health through this ban for e-cigarettes for those who are aspiring to quit the tobacco use based on the other preferences such as e-cigarettes. So they have insisted Union Minister to govern the e-cigarettes rather prohibiting it.[22]

1.6. Banning safer alternatives and not the regular cigarettes

There comes a point which imposes thoughts such as Banning of safer alternates but not the combustible cigarettes where the studies have shown that e-cigarettes are used as an effective mode to reduce smoking and are less addictive when compared to traditional cigarettes. In this instance, many social health experts have argued against the ban on e-cigarettes which appropriately makes no sense when the combustible cigarettes are readily accessible in the market.[22]

1.7. Hazards and benefits of e-cigarettes

E-cigarettes have gained popularity among the teens due to their easy accessibility, distinct flavors used and the idea of being safe when compared to cigarettes. All this consideration will surrender a teen towards smoking cigarettes at a peak risk in future.

The nicotine which is present in the e-liquids is easily absorbed by the blood which stimulates the adrenal gland for the release of epinephrine which is involved in the stimulation of central nervous system and further increases heart rate and breathing. A study suggested that e-cigarette products contain toxic chemicals as well as toxic metal nanoparticles which are found to be carcinogenic.[1]

E-cigarettes are considered to be a smoking cessation device. It has less number of toxic chemicals and said to be less harmful than conventional smoking. It acts as a tool which helps in quitting traditional smoking which can be outlined as e-cigarettes benefits. E-cigarettes provides benefit to adult smokers who are not pregnant where it acts as alternative for conventional smoking.[23]

2. Conclusion

Overall the discussion is about the controversy of e-cigarettes with respect to the device, its market, regulation norms of e-cigarettes at different platforms and amidst the debate over e-cigarettes banning in India. Varying point of views from experts, scientists, users with respect to e-cigarettes has been addressed which shares a mix opinion with the supporters promoting ban as well as the antagonist with the concept of regulating the e-cigarettes in India. The regulations which will be proposed for e-cigarettes must be suitable and convenient showing a competitive dominance in contrast to tobacco. Between the safety as well as the acceptability of use there should be harmonization in order to accomplish benefits pertaining to public health. Banning safer alternatives and not the conventional cigarettes is the most standard question which arises when coming to the point of banning of e-cigarettes in India and makes no sense until or unless regular cigarettes are banned. However, the government's definite conclusion recognizes that the benefits nor hazards of the constant use of e-cigarettes are unknown to everyone.

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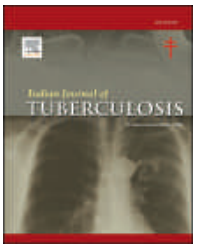

Review article

Metformin: A review of its potential as enhancer for anti tuberculosis efficacy in diabetes mellitus-tuberculosis coinfection patients

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ABSTRACT

Metformin is the most commonly prescribed drug for type 2 diabetes mellitus. Nowadays metformin is also use for efficacy in diabetes mellitus-tuberculosis coinfection patients through several mechanisms, such increasing superoxide production therefore activation isoniazid is increasing; inducing adeno-monophosphate kinase (AMPK) associated autophagy process; and regulating inflammation cytokines. This article will review the mechanism of action of Metformin as enhancer for anti tuberculosis efficacy.

1. Tuberculosis and its problems

Tuberculosis (TB), the infection caused by *Mycobacterium tuberculosis* (M.tb), remains a problem to overcome in Indonesia. In East Java Provence Indonesia 2015, the incidence of new tuberculosis (TB) cases reached 647 from 100,000 population. This increased nearly 2 times from the previous year incident, in 2014, that only 399 new TB cases from 100,000 population.[1,2] This phenomena was similar to TB incidence in the world. According to the World Health Organization (WHO) data in 2013, it states that the incidence of new TB cases in the world has increased 50%, and therefore WHO has declared for TB as a "global health emergency".[3,4]

Diabetes mellitus (DM) is one of the risk factors for TB infection. The risk ratio (RR) of TB infection in DM was increased 2.39 times and the risk of failure in TB treatment was also increased 1.69 times.[5–7] Hyperglycemia condition in DM patient could interfere the human's immune response due to decrease of 1) microvascular response to inflammatory mediators such histamine and bradykinin release; 2) mast cell degranulation; 3) interaction of leukocyte and other endothelial cells; 4) release tumor necrosis factor (TNF)-α, interleukin (IL)-1β dan prostaglandin (PG)-E2[8].

M. tuberculosis has ability to manipulate both innate and adaptive immune response, and called TB's escape mechanism. In this mechanism M.tb has high ability to avoid intracellular killing process and macrophage phagocytosis.[9] Hyperglycemia condition increases its ability in escape mechanism.[10] Therefore failure of TB treatment in DM has increased.[5–7]

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The aim of TB treatment is to cure patients, to prevent death, to prevent TB infection recurrence, to break the chain of transmission, and to prevent the occurrence anti tuberculosis resistance. However, M. tb is an acid-resistant, with its ability to growth very slow but resistance emerges very quickly when M.tb exposed only to one drug, therefore TB treatment was drugs combination of several mechanism.[11,12] The aims of drugs combination as anti tuberculosis were 1) increasing bactericidal activity since the beginning of TB therapy; 2) preventing drug resistance; and 3) improving the elimination process of M. tb in infected areas.

Anti tuberculosis is classified into two lines, first and second lines. The first line of anti tuberculosis are rifampicin, isoniazid, pyrazinamid, ethambutol and streptomycin. This first line group shows high effectiveness with acceptable toxicity and are given for at least 6 months.[11,12] However drug induced hepatitis (DIH) often occurs during the first line of TB therapy, and also other complications such gastrointestinal intolerance, rash, and renal failure.[11–13] The second-line of TB treatment such fluoroquinolones, aminoglycoside and others are given for multi-drugs resistances (MDR) for 18–20 months.[11,12,14] In this review, the focus is for first line of anti tuberculosis efficacy. Isoniazid (INH) is the highest inhibition activity, due to its bacteriostatic mechanism, at the beginning of TB treatment. INH inhibits M.tb (in vitro) at concentrations of 0.025–0.05mg/L, and it become more effective when combined with ethambutol, rifampicin, pyrazinamide and streptomycin.[11,12] Rifampicin (RIF) has the highest ability in M.tb elimination at the concentrations of 0.06–0.25 mg/L. However, both of INH and RIF prevalence of drug resistance are high.[11,12,14–16] The prevalence of RIF resistance occurs at 1 in each 10^7–10^8 CFU M.tb, and INH occurs at 1 in each10^6 CFU M.tb.[11] The M.tb resistance occurs through several mechanisms, such:1) The inability of anti tuberculosis to penetrate into M.tb cell walls due to its contain a lot of lipopolysaccharide and mannose[11,17]; 2) Anaerobic conditions cause M.tb to become dormant so that anti tuberculosis, such RIF unable to inhibit the metabolic process efficiently; 3) Changes in enzymes responsible for activating pro-drugs, such pyrazinamide (PYR) and INH; 4) Gene Mutase, such single point mutations of *pncA* gene in PYR, and *emb*B gene in Ethambutol.[11]

TB drug-resistant becomes a major bottleneck problem in TB infection control and eradication. It was estimated 480 000 new cases of MDR TB, and 210 000 deaths in 2013.[14,18] Extensively drug-resistant TB (XDR TB) has been reported from several countries and an estimated 9.6% of MDR TB patients are characterized as XDR TB that suffers from poor treatment outcomes.[14,19]

2. Host-directed therapy for TB patients

To develop an optimal therapeutic strategies, it needs proper understanding of TB infection pathogenesis, host-immune response, and escape mechanism. Uncontrolled chronic hyperglycemia condition in DM patients increase the risk of TB treatment failure, relapse of TB infection, and associated with death from TB infection.[20,21] The aim of new host-directed therapies identification, as WHO priority, is improving the clinical outcomes of TB patients.[22,23] Therefore, it could use to 1) shorten the duration of TB treatment; 2) prevent resistance and reduce lung tissue damage, through increased autophagia and other antimicrobial peptide production; 3) changes in macrophage effector mechanisms and modification of specific mechanisms, then preventing lung inflammation and matrix destruction; and 4) act on immunity regulation.[23] One of those drugs that has been known as host directed therapy is metformin (MET).

3. Mechanism of action of metformin as candidate for host-directed therapy in patients with diabetes mellitus – tuberculosis coinfection (Fig. 1)

Metformin(MET) is the most commonly prescribed drug for type 2 diabetes mellitus. MET through in silico studies, in vitro studies and in vivo studies using animal models, expressed as important role for anti tuberculosis through immunomodulatory mechanism,[24–26] as it is seen in Fig. 1

4. Metformin dan Superoxide Dismutase (SOD)

Superoxide Dismutase (SOD) is an enzyme produced in the host's antioxidant defense system.[27,28] TB infection increases reactive oxygen species (ROS) as respiratory burst result in macrophage phagocytosis process against M.tb. Excessive production of ROS associated with Th1 overactivation. Inhibits macrophage activity, and increasea tissue damage due to. Hyperglycemia condition could increase ROS production, therefore SOD levels could also increase also in DM patients.[29] KatG gene activates INH from prodrug to active drug. Apparently, SOD contributes during this mechanism, higher SOD related to better of INH's in inhibiting M.tb.[30] MET has ability in improving SODlevel during inflammation.[31,32] Based on this, the addition of MET provides synergism effects to increase the effectiveness of INH. MET has also a synergistic effect with RIF through increasing the expression of organic cation transporter (OCT) –1. The OCT-1 expression plays a role in inhibiting transcription M.tb.[25,33] Moreover, target of glycemic level for DM-TB patients is also need to be adjusted, therefore synchronized with SOD production.[34]

5. Metformin induced autophagy

M.tb has an escape mechanism through inhibition of host macrophage cells' autophagy.[9,17,35] Improving the autophagia process will improve anti tuberculosis in eliminating M.tb. MET activates Adeno Monophosphate Kinase (AMPK) and subsequent phosphorylation of unc-51-like kinase 1 (ULK1),[36] then AMPK works as mTOR inhibitor and enhances autophagy.[23,24,26,37] Therefore, MET from

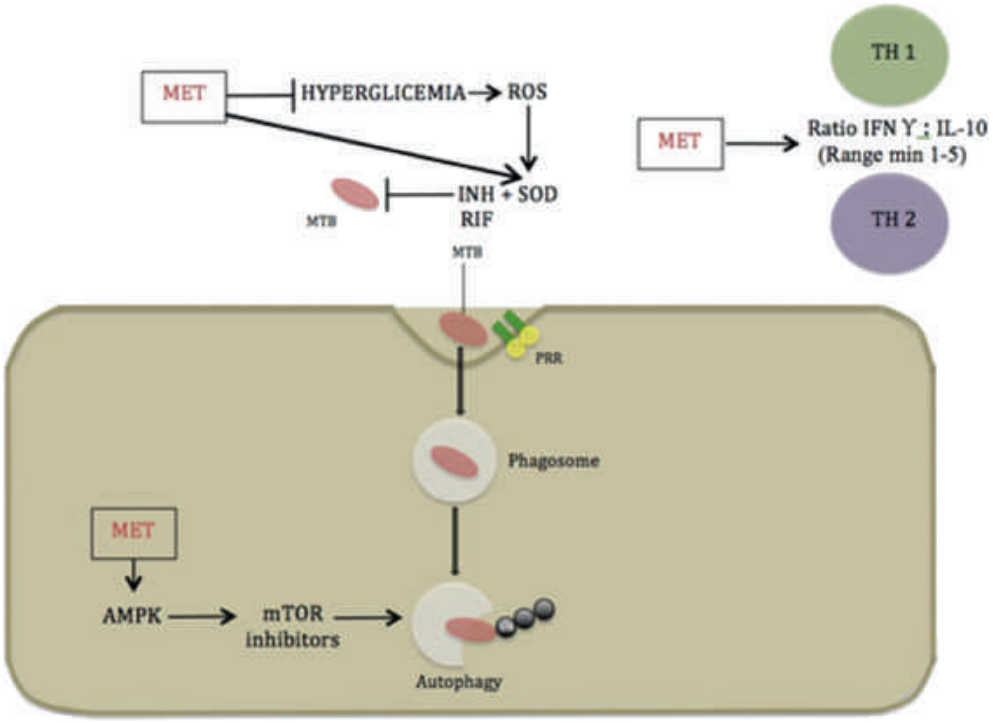

Fig. 1 – Mechanism of Action Metformin as Adjuvant therapy for DM-TB coinfection patients.

pharmacodynamics aspect has no effect to M.tb but works on host immune regulation.[34,38]

6. Metformin, interferon gamma (IFN-γ), interleukin (IL)-10 and its ratio

IFN-γ increases in chronic TB infection as the body's cellular immune response. Currently, IFN-release assay (IGRA) is used as a diagnostic tool for latent TB infection and as an indicator of therapeutic success in active TB infectionf.[39–41] IL-10 is a negative feedback regulator on the immune response produced by Th2 to inhibit excessive production of pro-inflammatory cytokines. IL-10 barriers the macrophage function, due to suppression of MHC class II molecules and reduces co-stimulator expression.[42–44]

MET associated AMPK activation, through thioredoxin-interacting protein (TXNIP) decreases activation of inflammatory mediators and transcription factors, including NF kappa B which encodes proinflammatory mediators45,46. In addition, in intracellular infections such TB MET through AMPK is also stimulated macrophage autophagy34,36, therefore MET accelerates M.tb elimination process without excessive inflammatory processes that can damage the tissue47.

7. Side effects of metformin that might occur

Gastrointestinal disorders (anorexia, nausea, vomiting and diarrhea) is one of the most common MET's side effect. Impaired absorption of vitamin B_{12}, impaired liver and orkidney function or in elderly people.[11,12] Increased levels of lactate or known as Metformin-associated lactoacidosis (MALA) although the occurrence is low, must still be prevented. MALA is a life-threatening event.[45] However, in Diabetes Tuberculosis coinfection patients, MALA could be prevented by determining patients criterias, including: 1) has minimal - moderate advanced pulmonary lesions in X-ray chest examination; 2) has oxygen saturation has at least above 92%; 3) has normal liver function (SGOT, SGPT) and normal kidney function (BUN, SK). Providing consultation, information and education related to the symptoms of lacto-acidosis is also needed during MET additional therapy. MET may increases lactate but rarely increase the risk of DM-TB coinfection patients experience MALA.[46,47] Therefore, MET is relatively safe to use for DM-TB coinfection patients.

8. What we will do in the future?

Developing new drug in host directed therapy that has similar function to MET is a prospective project. Enhancing anti tuberculosis efficacy through host immune response is future approach in TB elimination. MET has several potential effect in enhance anti TB, its side effect remains unpleasant.

Conflicts of interest

The author has none to declare.

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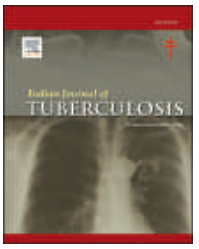

Review article

Prevalence of anemia among patients with tuberculosis: A systematic review and meta-analysis

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ABSTRACT

Introduction: Anemia is one of the most common hematologic problems occurs among patients with tuberculosis (TB). Many studies have been carried out estimating the prevalence of anemia among TB patients in different countries reported various results. This study aims to estimate the combined estimate of the anemia prevalence among these patients using systematic review and meta-analysis.

Methods: Required primary studies were provided after a comprehensive and systematic search in PubMed, Scopus, Science direct, Web of Science and also Google scholar search engine. These studies were then quality assessed using Newcastle–Ottawa Scale checklist. Random effects model was applied for combining the point prevalence with 95% confidence intervals.

Results: Of 41 papers entered into the meta-analysis, prevalence (95% confidence interval) of anemia among all TB patients as well as men and women were 61.53% (53.44–69.63), 66.95% (51.75–82.14) and 72.67% (60.79–84.54) respectively. Prevalence (95% confidence intervals) of mild, moderate and severe anemia were 35.67% (27.59–43.46), 31.19% (25.15–37.24) and 11.61% (7.88–15.34) respectively. In addition, prevalence (95% confidence intervals) of chronic disease anemia and iron deficiency anemia were 49.82% (15.58–84.07) and 20.17% (6.68–33.65) respectively.

Conclusion: Prevalence of anemia among TB patients was high especially among women. More than 43% of these patients suffered from moderate and severe anemia and about half of them had chronic disease anemia.

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1. Introduction

Anemia is a global concern affecting 1–8 percent of the world population.[1,2] Various hematologic problems have been diagnosed among TB patients such as different types of anemia, folate deficiency and sideroblastic disease.[1,3] Anemia has been observed among 32%–94% of these patients[4] according to various studies conducted in different countries.[2,5,6] Previous evidences showed that anemia during tuberculosis can be associated with higher mortality[1,2,6] and poor response to treatment.[1] Prevalence of anemia among TB patients is higher than that in general population.[2]

There is a proven interaction between TB and nutrition indicating the influence of TB on the nutritional status and influence of nutrition on the clinical manifestations of tuberculosis.[4] Despite the strong association between tuberculosis and anemia, no exact mechanism has been detected for occurrence of anemia among TB patients. It has been reported that the anemia can be induced due to inflammation as well as iron deficiency both of which are more common in developing countries.[3,7] Prevalence of anemia among TB patients in the studies carried out in India and Tanzania has been reported as of 72.7%[3] and 86% respectively.[2]

Among TB patients of many countries, prevalence of anemia has been reported with a considerable heterogeneity. Systematic review and meta-analysis is one of the suitable methods which can aggregate various evidences regarding a single subject and combine the primary results to estimate the most reliable pooled evidence for answering the question about the mentioned subject. This study applied this method to estimate the prevalence of anemia among TB patients.

2. Materials and methods

2.1. Search strategy

In this systematic review, available databanks such as PubMed, Scopus, Science direct, Web of Science as well as Google Scholar search engine were searched using the following keywords (Mesh or non-Mesh terms) and suitable operators (AND, OR, NOT):

(Tuberculosis OR Latent Tuberculosis OR Pulmonary Tuberculosis OR Pulmonary OR Extra Pulmonary Tuberculosis OR lung Tuberculosis OR Acid fast bacilli OR Mycobacterium tuberculosis) AND (Anemia OR Blood Cell OR Hemoglobin OR Iron OR Iron-Deficiency OR Infectious Anemia).

The search was conducted during 1–20 January 2018. In addition to the databank results, the list of references of the primary studies was investigated for finding any probable related evidence.

2.2. Inclusion criteria

The eligible papers that were published from any time to the end of 2017. According to PICO criteria, these studies should be carried out among TB patients (P), prevalence of anemia and also type and its severity were the primary and secondary outcomes of interest (O), the studies should be cross sectional or cohorts (and also case control studies in which cases were representative samples of all TB patients) and should report prevalence of anemia. It should be noted that "intervention" and "comparison" were not addressed as the inclusion criteria.

2.3. Study selection

At first, duplicate studies were identified and excluded using endnote software. Then, titles, abstracts and full texts of the studies were reviewed and irrelevant papers were removed.

2.4. Quality assessment

Risk of bias for each study was assessed using The Newcastle–Ottawa Scale checklist (NOS).[8] This checklist assigned between zero to seven scores to each study based on three sections (selection section with 3 scores, comparability with 2 scores and outcome with 2 scores) in the present meta-analysis. Studies achieved score less than four were excluded from the meta-analysis. Quality assessment was conducted independently by two methodologists.

2.5. Data extraction

Required data for meta-analysis including title of the paper, first author identities, year and country in which the study carried out, type of the study, total sample size as well as sample size of men and women, target population, prevalence of anemia and its types (chronic disease, iron deficiency, megaloblastic, dimorphic) and severity (mild, moderate and severe) based on genders were extracted and transformed into excel software.

2.6. Analysis

Stata version 11(Stata Corp, College Station, TX, USA) was used for statistical analysis. At first, standard error for the prevalence was calculated according to binomial distribution formula. The heterogeneity between the results of the studies was assessed using Cochrane test and I-square indicator. If p-value was less than 0.1, the results was considered statistically significant. The I-squared was classified as low (less than 25%), moderate (25%–75%) and high (more than 75%) according to Higgins criteria.[9] Because of high heterogeneity observed between the results of the primary studies, random effects model was applied for combining the results. Point and pooled prevalence of the anemia and its types and severity with 95% confidence intervals (CI) were illustrated by forest plots. Investigating the variables affecting the heterogeneity was conducted by meta-regression models. Moreover, publication bias was assessed using Egger test.

3. Results

At first, 756 evidences was identified which reduced to 549 studies after exclusion of the duplicates. Following screening of titles/abstracts, 66 papers were remained. Full texts of these articles were reviewed and 41 relevant papers were

selected.[2–6,10–45] Some of the selected evidences were abstracts of papers presented in seminars and were published in journals including required information for meta-analysis.

Risk of bias analysis showed that all 41 selected articles had at least four scores made them eligible for participating in the meta-analysis. Of them, 40 studies reported total prevalence of anemia while Seung study[28] reported just prevalence of severe anemia. These studies had been carried out in 21 countries (Fig. 1).

The total prevalence of anemia among 16671 TB patients recruited in these 40 studies was estimated as of 61.53% (95% CI: 53.44–69.63). The heterogeneity between the results of these primary studies was statistically significant (Table 2, Fig. 2). According to the results of meta-regression models, country where the study conducted did not play a significant role in the heterogeneity ($\beta = 0.29$, $p = 0.703$). Egger test showed no evidence of publication bias ($\beta = 3.25$, $p = 0.391$). Prevalence of anemia among 1635 male TB cases had been reported in 11 studies. Pooled prevalence (95% CI) of anemia among these patients was estimated as of 66.95% (51.75–82.14) (Fig. 3). Prevalence of anemia among 1742 female TB cases was reported in 11 studies and pooled estimate was estimated as of 72.67% (95% CI: 60.79–84.54) (Fig. 4).

The total prevalence (95% CI) of mild (10 studies including 3662 patients), moderate (11 studies including 4070 patients) and severe anemia (11 studies including 3797 patients) was estimated as of 35.67% (27.59–43.46), 31.19% (25.15–37.24) and 11.61% (7.88–15.34) respectively (Fig. 2).

Prevalence of chronic disease anemia had been reported in three studies with total sample size of 508 TB patients. The pooled prevalence (95% CI) of anemia among these patients was estimated as of 49.82% (15.58–84.07). Prevalence of iron deficiency anemia among 1890 TB patients had been reported in seven studies. The combined prevalence (95% CI) of this type of anemia was estimated as of 20.17% (6.68–33.65) (Table 2). Prevalence of megaloblastic and dimorphic anemia among TB patients had been estimated just in the study conducted by Mukherjee et al in India as of 12.5% and 2.2% respectively (Table 1).

4. Discussion

Our meta-analysis showed that prevalence of anemia among all TB patients as well as among male and female cases was 61.53%, 66.95% and 72.67% respectively. In addition,

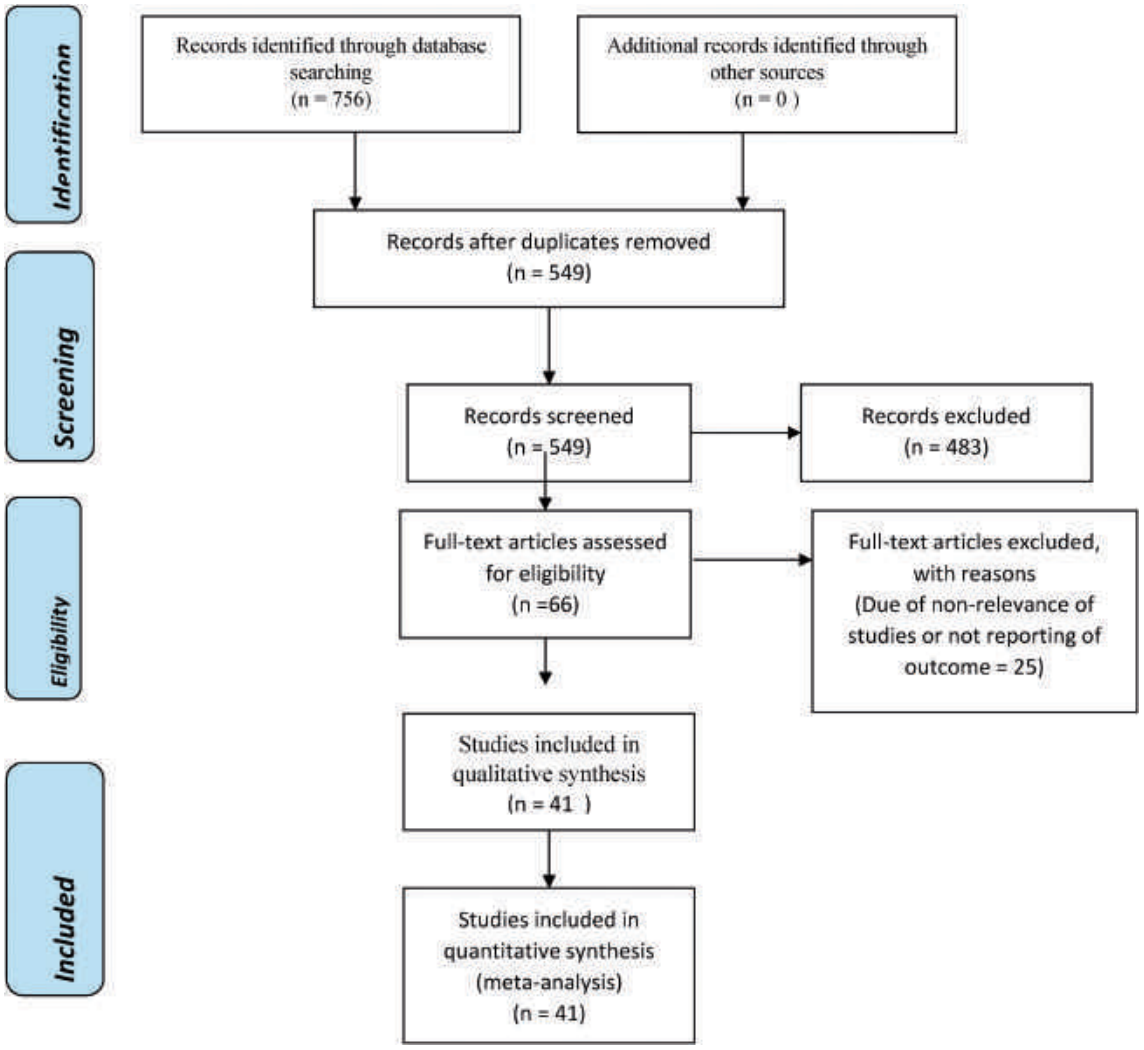

Fig. 1 – Searching and selection of the primary studies.

Table 1 – Characteristics of primary studies included in the meta-analysis.

First author, Publication year, Country study	Sample size			Prevalence of Anemia by gender			Prevalence of anemia by severity			Prevalence of Anemia by type				Risk of bias score
	Total	Male	Female	Total	Male	Female	Mild	Moderate	Sever	Chronic-disease	Iron deficiency	Megaloblastic	Dimorphic	
Yoon, 2004, Korea [10]	312	149	163	50.3										5
Lee, 2004, Korea [11]	897			32.7										5
Mulenga, 2017, Congo [12]	200	130	70	69.5	69.23	70	65.5		4					6
Kwon, 2016, Korea [13]	2481	1417	1064	27.4										6
Hungund, 2012, Belgaum[14]	100			96			50	37	9					5
Monjur, 2014, Bangladesh (15)	121	66	55	75.2	71.21	80								6
Al-Jahdali, 2000, Saudi Arabia (16)	47	30	17	66										5
Minchella, 2015, Gambia[17]	45	28	17	57.77							44.44			6
Babamahmoodi, 2015, Iran[18]	212	131	81	80										6
Dileepan, 2017, India[19]	200	125	75	75.5	67.2	89.33	50.5	17.5	7.5					5
Oliveira, 2014, Brazil[4]	166	126	40	89.15	92.06	80				75.9	2.4			6
Adzani, 2016, Indonesia[20]	49	26	23	63.26			36.73	26.53						5
Isanaka, 2011, Tanzania[6]	684			64							58			6
Yaranal, 2013, India[21]	100	71	29	74	71.83	79.31								5
Tsao, 2001, Taiwan[22]	1826	1298	528	66										6
Mukherjee, 2017, India[3]	242	182	60	72.7	73.6	70	44.2	19	9.5	58.5	26.7	12.5	2.2	6
Karyadi, 2000, Indonesia[23]	41	25	16	58.53										6
Kayabasi, 2008, Turkey[24]	21	11	10	71.42										4
Nagu, 2014, Tanzania[2]	1245	831	414	86			29.71	43.53	12.45					6
Lee, 2006, Korea[5]	880	472	408	31.93	28.17	36.27								6
Shu, 2012, Taiwan[25]	91	55	36	70.32										6
Sahiratmadja, 2007, Indonesia[26]	378	225	153	63.22										6
Alavi-Naini, 2013, Iran[27]	715	375	334	25.6										6
Seung, 2009, South Africa[28]	76	40	36						24					6
Ramírez-Lapausa, 2015, Spain[29]	21			9.5										6
Bashir, 2015, Sudan[30]	100	77	23	44						15	7			6
Van Lettow, 2005, Malawi[31]	500	227	273	85.2				26.2			5			6
Kerkhoff, 2015, South Africa[32]	174	59	115	84.48			15.51	37.35	31.6					6
Chung-Delgado, 2011, Peru[33]	720	446	272	15.27										6
Cegielski, 2012, United States[34]	61	24	37	11.4							0			6
Kassa, 2016, Ethiopia[35]	168	95	73	54										6
Kerkhoff, 2016, South Africa[36]	153	53	100	85.62	83.01	87	15.03	37.9	32.68		1.3			6
Westreich, 2009, South Africa[37]	1197	502	695	72.18										6
Van Rie, 2011, South Africa[38]	501	224	277	72										6
Kerkhoff, 2014, South Africa[39]	485	177	308	55.46	42.94	62.66	24.95	25.36	5.15					6
Atomsa, 2014, Ethiopia[40]	108	45	63	37.03				35.18	1.85					6
Agarwal, 1977, United States[41]	16	13	3	81.25	84.61	66.66								4
Kerkhoff, 2014, South Africa[42]	814	220	594	70.51	55.45	76.09	28	38.08	4.42					6
Hussain, 2004, Pakistan[43]	110			62										6
Banerjee, 2015, India[44]	227	179	48	68.28										5
Mert, 2017, Turkey[45]	263	142	121	86										6

Table 2 – Pooled estimates of anemia prevalence among TB Patients.

Variables	Number of primary studies	Sample size	Pooled prevalence (based of Random effect model)		Heterogeneity		
			%	CI 95%	I-square (%)	Q	P-value
Anemia among total patient	40	16671	61.53	53.44–69.63	99.3	5823.01	<0.001
Anemia among male patient	11	1635	66.95	51.75–82.14	98	496.34	<0.001
Anemia among female patient	11	1742	72.67	60.79–84.54	96.4	281.04	<0.001
Mild anemia	10	3662	35.67	27.59–43.46	96.3	244.25	<0.001
Moderate anemia	11	4070	31.19	25.15–37.24	93.8	160.99	<0.001
Severe anemia	11	3797	11.61	7.88–15.34	94.4	179.53	<0.001
Anemia of chronic disease	3	508	49.82	15.58–84.07	98.8	163.39	<0.001
Anemia of iron deficiency	7	1890	20.17	6.68–33.65	99.3	853.93	<0.001

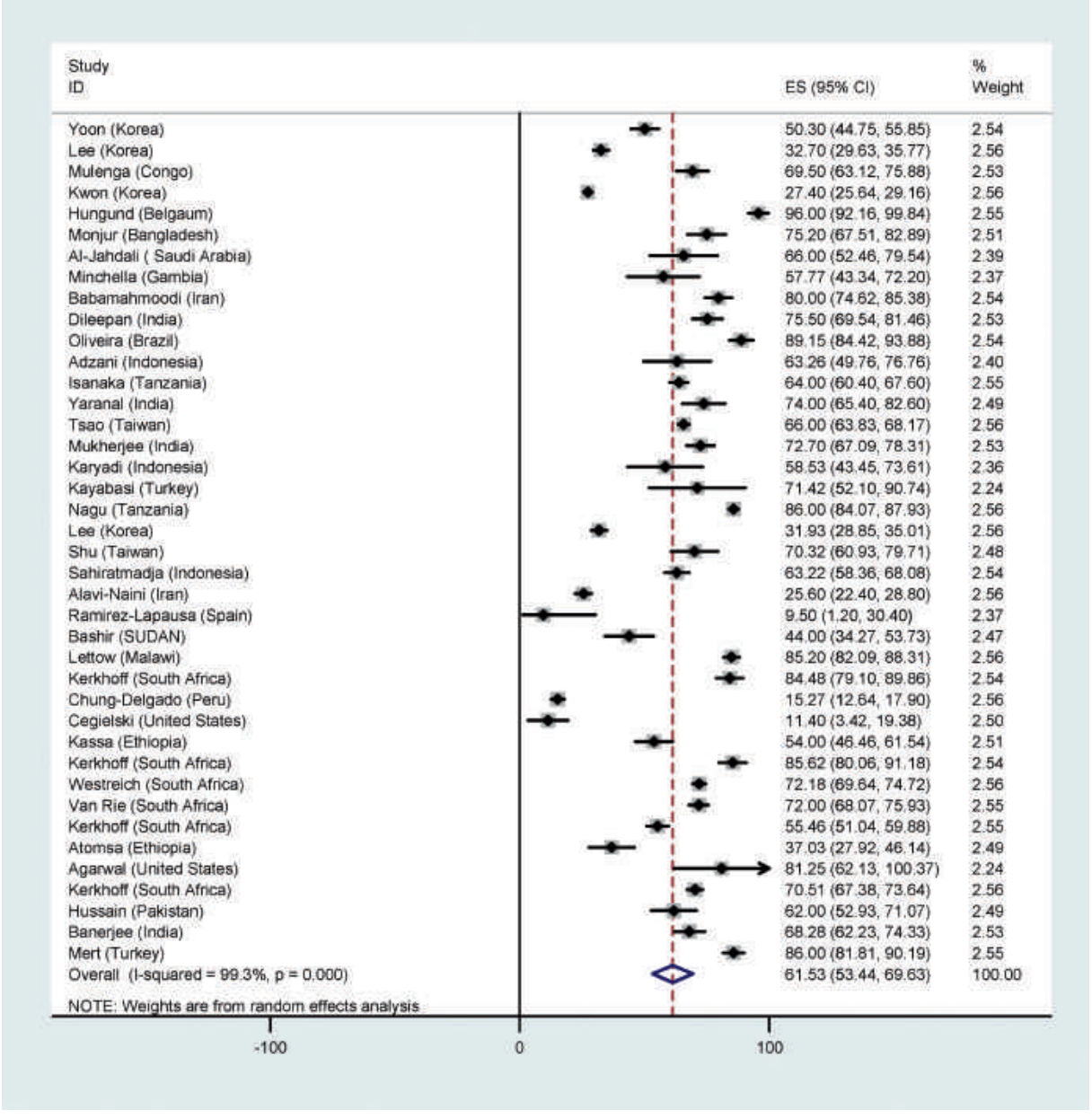

Fig. 2 – Pooled prevalence of anemia among TB patients.

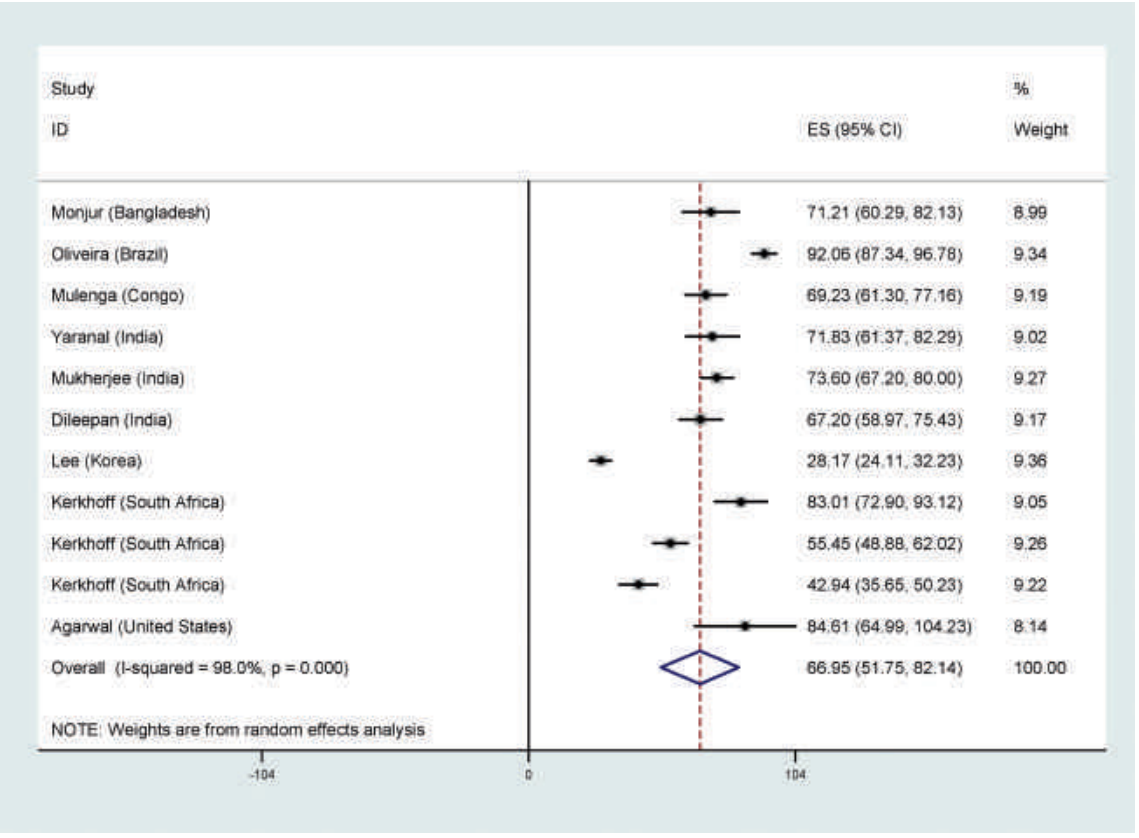

Fig. 3 – Pooled prevalence of anemia among male TB patients.

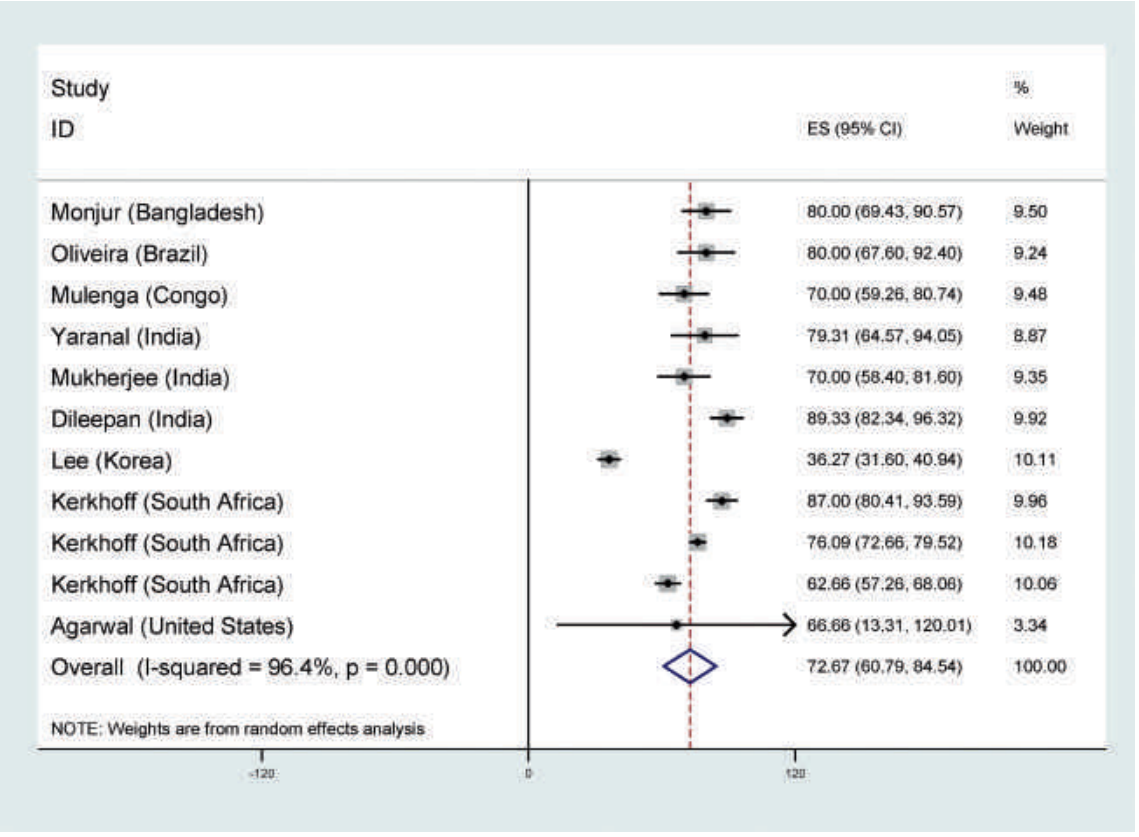

Fig. 4 – Pooled prevalence of anemia among female TB patients.

prevalence of mild, moderate and severe anemia among these patients was 35.67%, 31.19% and 11.61% respectively. Moreover, 20.17% and 49.82% of patients with tuberculosis had respectively iron deficiency anemia and chronic disease anemia.

Lee et al reported that 31.9% of TB patients in South Korea suffered from anemia[5] while 69% of TB patients in Congo had anemia 92.2% of whom had severe anemia and iron deficiency was observed among 48% of cases.[12]

In Brazil, Oliveira et al showed that prevalence of chronic disease and iron deficiency anemia was 75.9% and 2.4% respectively. They also found that the anemia was significantly associated with male gender, weight loss, low MCV, high RDW, high serum ferritin and high ESR.[4]

Another study was conducted by Mukherjee among 242 TB patients of a hospital in Uttarakhand, India reported 72.2% anemia among these patients 44.2% of whom were mild. They also showed that 58.8%, 26.7%, 12.5% and 2.2% of TB patients were suffered from chronic disease anemia, iron deficiency, metaloblastic and dimorphic anemia.[3]

Of 85 TB patients investigated in Iran by Eishi et al, 53% had anemia 21.2% of whom had severe anemia. The anemia was significantly lower after treatment.[46] In Tanzania, of 1245 TB cases, 86% had anemia.[2]

Therefore, we can conclude that anemia is a common hematologic disorder among TB patients which can influence the treatment result. Therefore, screening of TB patients regarding anemia at the beginning of treatment and more attention to the anemic cases during treatment is of great importance. Sustainable development goals indicated 35% reduction in TB death rate and 20% reduction in TB incidence until 2020.[47] One of the main strategies for TB control and achievement of these SDGs is successive treatment of smear positive TB patients. Remaining sputum smear positive during treatment is a predictor of adverse consequences such as death, treatment failure and drug resistance. Moreover, it facilitates the development and spreading cycle of the disease especially drug resistant cases within the community.[48–51] Some evidences showed that anemia is one of the main factors associated with TB treatment failure.[2,41] Thus, it is necessary to consider these findings in the TB control program. It is also necessary to investigate all causes of anemia among TB cases.

The great heterogeneity of the populations investigated in the primary studies in terms of TB type, co-morbidity with other diseases and exposure to different risk factors, age, duration of the disease, history of previous anti-TB treatment and use of immunosuppressive drugs were the first limitation of the current research. Because of the limited number of the primary studies, it was not possible to analyze the results based on these subgroups. Socio-economic status of the countries and difference in prevalence of the anemia among general populations was the other limitation and source of the heterogeneity. In addition, the prevalence of anemia in each gender was higher than the total prevalence. The reason for such discrepancy is that total prevalence was selected from all 40 studies but gender specific prevalence was estimated based on the results of just 11 studies.

Despite the above mentioned limitations, this meta-analysis provided reliable and valuable evidences regarding the high prevalence of anemia among TB patients especially among men. More than 40% of TB patients sufferers from moderate to severe anemia and approximately half of them have anemia due to chronic diseases.

The results of the present systematic review-meta-analysis suggests that the future research policies focus mainly on the cohort studies and not cross sectional researches which do not investigate all aspects of the problem. Cohort studies can provide reliable information in term of the real causal association between anemia and tuberculosis. In the field of health policy making it is recommended to add CBC tests to the routine laboratory investigations in TB cases before treatment and special follow up of patients with anemia. Finally, in the field of general policy making, it is necessary to establish special programs for improvement of the nutritional status of the communities as well as TB cases especially in developing countries.

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.2019.04.002.

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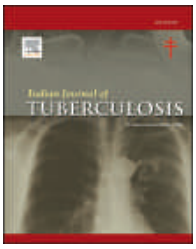

Correspondence

Limitations of CBNAAT for diagnosis of mycobacterium tuberculosis in gastric aspirate and pleural fluid

Sir,

Cartridge based nucleic acid amplification test (CBNAAT) is now being used to diagnose pulmonary tuberculosis (PTB) and Extra-pulmonary tuberculosis (EPTB) in India for more than 6 months. The kits are being provided by the Revised National TB control Program of India. The indications for EPTB testing include any suspected case of EPTB that includes pleural effusion (PLEF), ascitic fluid, cerebrospinal fluid, pericardial fluid, lymph node aspirate, pus, or fine needle aspiration (FNA) from solid organs.[1] We did an analysis of 6 months data generated in our hospital through the CBNAAT tests done to detect PTB and EPTB.

In six months from 23rd of February, 2016 to 30th of July 2016, 581 samples were tested using CBNAAT for mycobacterium tuberculosis (MTB). Out of these 151 (25.9%) were MTB positive, 50.3% PTB and 49.6% EPTB [Table 1]. The overall positivity rate for symptomatic presumptive PTB was 25.6% and for presumptive EPTB was 26.4%.

Presumptive PTB: Total samples were 297; including sputum 225(75.8%), gastric aspirate (GA) 53 (17.8%), and bronchoalveolar lavage 19(6.4%) [Table 1]. Only sputum showed a high positivity rate (33.3%) while Gastric aspirate had zero positivity despite contributing 17.8% of all presumptive PTB samples. In addition, the bronchoalveolar lavage also had a poor positivity rate of 5% in the 19 samples tested. Of 53 presumptive cases of PTB in children whose GA samples were received 33 (62%) were males and 20 (38%) were females. Mean age of children was 4.7 years.

Presumptive EPTB: Total samples were 284; including lymph node aspirates 124 (43.6%), pleural effusion (PLEF) 86 (30.3%), pus 31(11%), solid organ Fine needle aspiration (FNA) 16 (5.6%) and others 27 (9.5%) [Table 1]. High positivity rate among presumptive EPTB was obtained for pus samples (54.8%) followed by FNA material (37.5%) and Lymph node aspiration (33.3%). Pleural fluid contributed 30.3% of all EPTB samples but the positivity rate was just 9%. Also the ascitic fluid, synovial fluid, knee aspirate and liver abscess had 0 positivity rate. The mean age of people with suspected tubercular effusion was 40.2 years. There was a preponderance of PLEF in males as 66 (77%) were males and 20 (23%) were females.

This is a report from programmatic setting which highlights the poor efficiency of CBNAAT in detection of some classes of TB using GA and PLEF. High positivity rate for pus samples is a good sign for the program but low positivity rate in GA may be a setback if we look at the efforts the TB control program in India is putting in detection of childhood tuberculosis. Each CBNAAT kit costs a lot to the program and the low yield of GA using CBNAAT in programmatic settings raises doubts about its sensitivity and efficiency in practical use for childhood tuberculosis. The WHO meta-analysis shows a sensitivity of 83.8% for GA. No doubt, when compared to other tests like culture of GA the CBNAAT is easy and has high sensitivity rate, but the positivity rate among the presumptive samples in programmatic setting is also an important parameter to be considered for its generalized use in future.[2] India is a resource limited country, as far as health is concerned. So, judicious use of test like CBNAAT is needed to give efficient results. Nil MTB in 58 GA samples is a sign of poor efficiency of the test for the program as found by our study among presumptive PTB cases in children. Similar area of concern is efficiency of CBNAAT for EPTB in PLEF. The meta-analysis by WHO report also shows poor sensitivity of 24.8–64.7% compared to PLEF culture in the controlled settings.[2] The Sensitivity was as low as 17% when compared to a composite standard that included biochemical tests like Adenosine Deaminase (ADA) and the response to treatment. [2] The positivity rate in our settings for PLEF was 9%. If we look at the bulk of samples tested for PLEF, the efficiency of CBNAAT for PLEF is highly questionable. Most of the PLEF patients that are not having overt heart failure will be diagnosed EPTB based upon the Light's criteria and ADA levels. They are put on ATT and they improve. So, a simple test like Adenosine ADA in comparison has very high sensitivity for tubercular PLEF. All patients are tested for ADA irrespective of their CBNAAT results, so doing CBNAAT just

Table 1 – Total numbers and positivity rate of various samples tested for Tuberculosis using Cartridge based nucleic acid amplification test.

Pulmonary tuberculosis		
Sample	**Number tested**	**Positive (Proportion)**
Sputum	225	75 (33.3%)
Bronchoalveolar lavage	19	1 (5%)
Gastric aspirate	53	0 (0%)
Total suspected PTB	**297**	**76 (25.6%)**
Extrapulmonary tuberculosis (EPTB)		
Sample	**Number tested**	**Positive (Proportion)**
Pus	31	17 (54.8%)
Fine needle aspiration material	16	6 (37.5%)
Lymph node	124	42 (33.8%)
Pericardial fluid	3	1 (33.3%)
Cerebrospinal fluid	8	1 (12.5%)
Pleural fluid	86	8 (9%)
Ascitic fluid	8	0 (0%)
Synovial fluid	3	0 (0%)
Knee aspirate	3	0 (0%)
Liver Abscess	2	0 (0%)
Total EPTB	**284**	**75 (26.4%)**

adds to the cost of the patient treatment without much advantage to the program.

Results were similar (nil MTB+) for other fluids like Ascitic fluid and synovial fluid aspirate. CSF, Pericardial fluid, Pus, FNAC, lymph node aspirate, and sputum however yielded better results on CBNAAT.

To conclude, use of CBNAAT for diagnosis of PTB through GA samples is not cost effective, though it may be time efficient compared to GA culture. Similarly use of CBNAAT for diagnosis EPTB in PLEF samples is also not cost effective more so, when all samples are also being subjected to ADA and other biochemical testing that yields better results than CBNAAT. We suggest that a larger analysis of ongoing program is needed where pooled data from all centers performing CBNAAT is analyzed for the efficiency of CBNAAT for GA and PLEF to diagnose PTB and EPTB respectively.

Limitations: Since this was an analysis of the records available in the microbiology lab, we cannot comment much on what alternative tests were used to make a diagnosis in CBNAAT negative patients. In the programmatic settings the samples sent for CBNAAT are not cultured routinely, unless specifically requested. So in case of CBNAAT negative result we don't have any reference method to compare the results of CBNAAT.

Author contributions

Vivek Chauhan: Contributed in design, analysis, and manuscript preparation

Suman Thakur, Rajesh Kumar Sood: Contributed in design, data collection, and manuscript preparation

Source of support

None.

Conflict of Interest

The authors have none to declare

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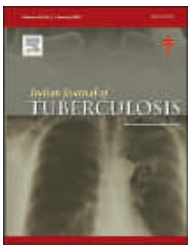

Case report

Primary hepatic tuberculosis masquerading as intrahepatic cholangiocarcinoma

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ABSTRACT

Abdominal tuberculosis is a common clinical entity in Indian subcontinent; however, hepatic tuberculosis in the absence of miliary abdominal tuberculosis is restricted to the case reports and small case series in English literature. It mimics common liver diseases like liver abscess and tumours. We report a case of 38 years old male presenting with abdominal pain, loss of appetite and weight initially misdiagnosed as intrahepatic cholangiocarcinoma on magnetic resonance imaging and FNAC of the lesion but later diagnosed as a case of hepatic tuberculosis on post operative histopathology specimen. It is important to consider tuberculosis in the differential diagnosis when suspecting lymphoproliferative or metastatic diseases in a patient with vague symptoms.

1. Introduction

Tuberculosis (TB) is known to involve the liver in different ways. Hepatic involvement in TB as part of disseminated TB is seen in up to 50–80% of cases. However, hepatic TB, particularly in the absence of miliary TB, is rare and represents less than 1% of all cases of TB.[1] Primary hepatic tuberculosis result from tubercular bacilli gaining access to portal vein from a microscopic tubercular focus in the bowel with subsequent healing taking place at the site of entry leaving no trace of it. Hepatic TB lacks typical clinical manifestations and can be difficult to differentiate from other malignancies such as hepatocellular carcinoma, intrahepatic cholangiocarcinoma (iCCA), klatskin tumour, and liver abscess using imaging modalities.

2. Case report

A 38-year-old male presented to our outpatient department with abdominal pain, loss of appetite and weight since two

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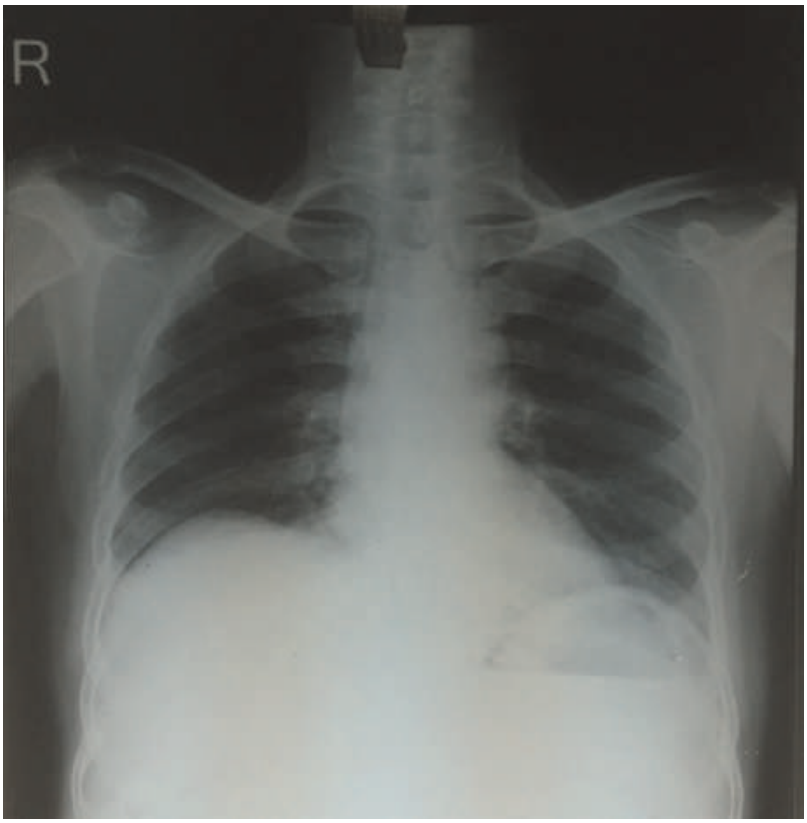

Fig. 1 – Chest X-ray PA view showing no pulmonary involvement.

months. There was no history of cough, fever or noticing yellowish discolouration of eyes. He was a recently detected diabetic and was on oral hypoglycemic agents. No history of hypertension, ischaemic heart disease, tuberculosis or kochs contact or jaundice in past. Patient was a chronic alcoholic and smoker and had left both addictions seven years back.

On general physical examination, patient was thinly built and had pallor. No signs of icterus, clubbing, cyanosis, pedal oedema or superficial lymphadenopathy. On perabdominal examination, there was fullness in epigastrium with presence of 6 × 6 cm nontender lump on palpation. Blood investigation revealed microcytic hypochromic anaemia with haemoglobin of 8 g%, total counts of 6400, differential counts of 62 neutrophils, 34 lymphocytes, 4 monocytes and 2 eosinophils. Erythrocyte sedimentation rate (wintrobes method) was 44 mm in the first hour. Fasting blood sugar was 208 mg%. Liver function tests (LFT) were deranged with total bilirubin of 2.75 mg/dl, serum aspartate transaminase (SGOT) 74 U/l serum alanine transaminase (SGPT) 55 U/l and alkaline liver phosphatase (ALP) of 232 IU/l. Sputum for AFB was negative. Chest skiagram showed no parenchymal opacity or medistinal lymph nodes (Fig. 1). Ultrasound abdomen showed a mass in left lobe of liver. So in view of this a multiphasic contrast enhanced computer tomography (CT) scan was done which revealed a large mass 11 × 7 cm involving almost entire left lobe of liver mainly segments II, III, IV with areas of enhancements (Fig. 2A). Ultrasound guided biopsy was done and revealed features suspicious of hepatocellular carcinoma yet tuberculosis could not be ruled out. Serum alpha fetoprotein (AFP), carcinoembryonic antigen (CEA) and CA 19.9 were within normal limits.

Because of this diagnostic dilemma, patient was referred to a higher centre for further management. He was further evaluated with magnetic resonance imaging (MRI) abdomen which showed features of iCCA (Fig. 2B). Upper gastrointestinal endoscopy did not reveal any varices or portal gastropathy. Patient underwent a middle hepatic vein sacrificing left hepatectomy. Intraoperative ultrasound did not show any satellite lesions. Final histopathology report showed necrotizing granulomatous inflammation with langhans type giant cells likely of kochs aetiology (Fig. 3). Special stains for acid fast bacilli (AFB), gomori methanamine silver, per-iodic acid Schiff were negative. It was decided to start patient on antitubercular therapy. But since liver functions were deranged, a daily regimen comprising nonhepatotoxic drugs i.e. injection streptomycin 0.75 g, tab Ethambutol 800 mg and tab Ciprofloxacin 750 mg was started. Patient was followed up with LFT's every fortnightly which started showing a decreasing trend. The patient started showing clinical improvement with decreasing abdominal pain and weight gain of 6 kg after two months of therapy. The patient is still undergoing chemotherapy and is doing fine.

3. Discussion

The clinical classification and nomenclature of hepatic TB is confusing in the literature.[2–5] It classified by Levine as miliary

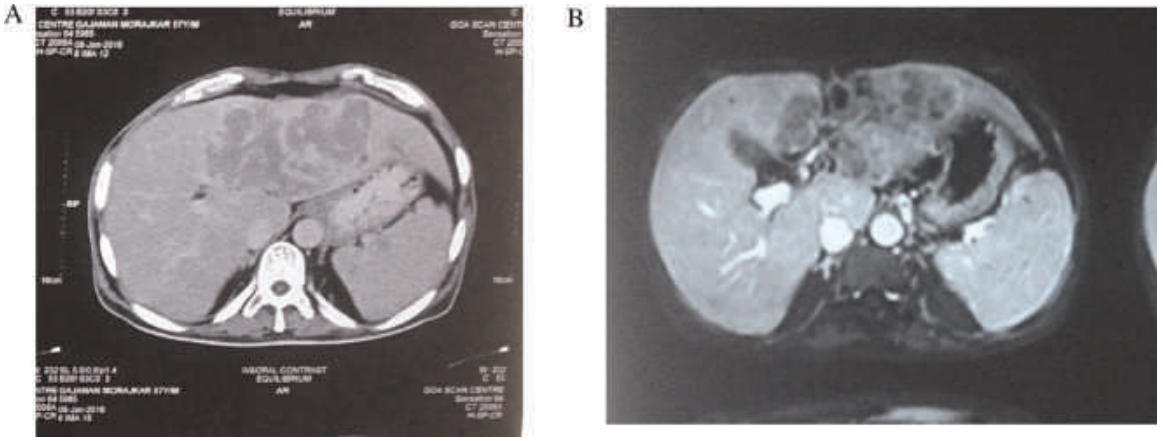

Fig. 2 – (A) CECT Abdomen showing a Nonenhancing mass 11 × 7 cm with slightly enhancing peripheral rim involving almost entire left lobe of liver mainly segments II, III, IV. As can be seen splenic parenchyma is not involved. (B) Magnetic resonance imaging (MRI) abdomen showing a hypointense mass with a hypointense rim in left lobe of liver on T1-weighted imaging.

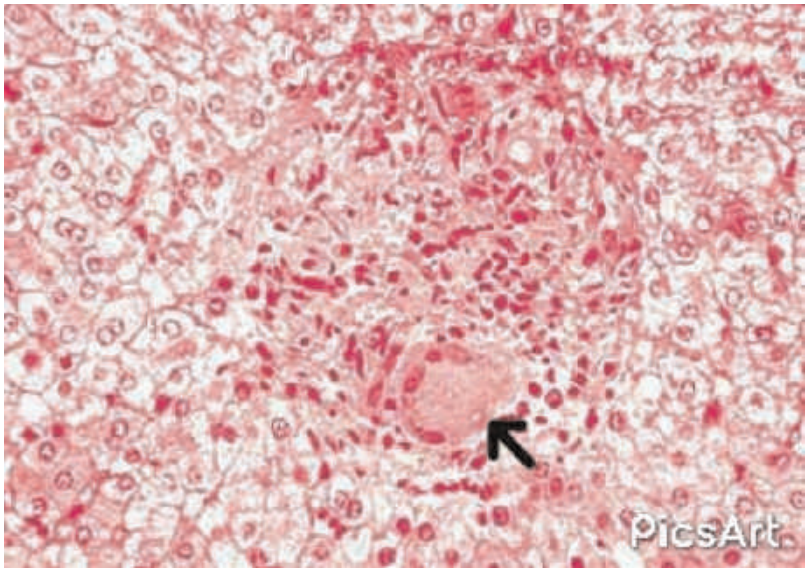

Fig. 3 – Postoperative histopathology report of liver mass showed caseation necrosis with epitheloid cells and langhans type giant cells (black arrow).

tuberculosis, pulmonary tuberculosis with hepatic involvement, primary liver tuberculosis, focal tuberculoma or abscess, or tuberculous cholangitis.[5] However, Reed divided it into three forms: tuberculosis of the liver associated with generalized miliary tuberculosis, primary miliary tuberculosis of the liver, and primary tuberculoma or abscess of the liver.[2]

The most common form of hepatic involvement is the miliary form of TB. It is believed that the route of transmission is different in miliary TB and primary hepatic TB. In the miliary form of TB, hematogenous dissemination of the bacteria seems to be the route of dissemination, while in primary hepatic TB, the tubercle bacillus reaches the liver through the portal vein from the intestine. Because low oxygen tension in the liver is unfavourable for mycobacteria growth, primary hepatic TB was observed in about 1% of hepatic TB cases.[6] On the other hand, hepatic involvement can be seen in nearly 70% of disseminated cases of TB.[1]

Hepatic TB lack typical clinical manifestations and imaging diagnosis, it may follow common clinical complaints with mild fever, right upper quadrant pain, hepatomegaly, weakness, night sweats, and so on. Hepatomegaly is usually found with an increase in alkaline phosphatase and normal transaminase levels.[2,3]

On CT scans, hepatic TB shows a nonenhancing, central, low density lesion owing to caseation necrosis with a slightly enhancing peripheral rim corresponding to surrounding granulation tissue. MRI of hepatic TB shows a hypointense nodule with a hypointense rim on T1-weighted imaging. T2-weighted imaging shows a hypointense, isointense or hyperintense nodule with a less intense rim.[6] However,

imaging features of hepatic TB might be multiple lesions of varying density, indicating that there are lesions in different pathologic stages coexisting in hepatic TB, including TB granuloma, liquefaction necrosis, fibrosis or calcification.[7] Therefore, it is very difficult to differentially diagnose using imaging modalities primary hepatic TB from iCCA. Hepatic TB also shows FDG avidity on F-18 fluorodeoxyglucose positron emission tomography (FDG PET)/CT similar to other malignant tumours.[8] F-18 FDG PET/CT is also less useful in differentiating hepatic TB from other hepatic necrotic masses because FDG-avidity can also been seen in necrotic tumours such as hepatocellular carcinoma, and metastatic carcinoma. Clinical manifestations, laboratory findings, and imaging studies are varied and nonspecific, so pathologic examination of liver lesions is essential to confirm hepatic TB. Therefore, percutaneous fine needle biopsy is an excellent diagnostic method.

In our case, we believed the hepatic mass was iCCA without doubt because it showed typical imaging features of iCCA. Moreover, according to the guidelines for the diagnosis and management of iCCA, a presumed radiographic diagnosis is sufficient in noncirrhotic patients in whom a decision has been made to proceed with surgical resection.[9] Our patient underwent an USG guided needle biopsy, and was preoperatively misdiagnosed with iCCA, and we later confirmed primary hepatic TB by postoperative pathology. It is most ideal to demonstrate AFB in the liver biopsy specimen, because AFB is most easily found in caseaous necrosis. However, the sensitivity of staining for AFB is low (0–45%) and culture is also low (10–60%). Polymerase chain reaction (PCR) directly detects the presence of *Mycobacterium tuberculosis*, which is more useful for diagnosis of TB, but the positivity rate is 57%.[5,10] For these reasons, the absence of AFB does not necessarily exclude TB, particularly in endemic areas of TB. Therefore, even without finding tubercle bacillus, the presence of a caseating granuloma in histologic examination is diagnostic. In our case, AFB smear test, culture, and PCR assay for *M. tuberculosis* were all negative.

In summary, primary hepatic TB is very uncommon, lacks typical symptoms, signs and laboratory findings. It is difficult to diagnose primary hepatic TB using imaging modalities because the imaging features of the hepatic TB are various and nonspecific. In patients with most frequent systemic manifestations of TB such as high fever, weight loss, right upper quadrant pain and hepatosplenomegaly; suspicion of hepatic TB for differential diagnosis is important especially in endemic areas and more so in patients having immunocompromised states like in our case who was a diabetic.

Conflicts of interest

The authors have none to declare.

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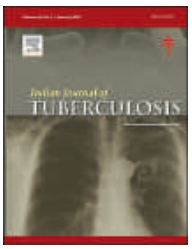

Case report

Concomitant presentation of sarcoidosis and pulmonary tuberculosis with ARDS: A diagnostic dilemma and therapeutic challenge

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ABSTRACT

Tuberculosis and sarcoidosis are chronic multisystem granulomatous conditions which have different aetiology and management but may mimic each other clinically, radiologically and pathologically. Both these diseases usually have a sub acute or chronic presentation and it is rather uncommon for them to coexist or present with acute respiratory failure.

We report a case of a 57-year-old male who presented with pyrexia of unknown origin with chronic cough. He was initially diagnosed to have sarcoidosis based on clinico-radiological and histologic evidence and was started on corticosteroids. However, he presented within two weeks with acute respiratory distress and on further investigation was diagnosed with co-existing pulmonary tuberculosis.

1. Case report

A 57-year-old male, previously healthy presented with high grade fever for 15 days associated with loss of appetite. There was history of cough with occasional expectoration. He had history of previously treated pulmonary tuberculosis during childhood. On examination, he was conscious and oriented, haemodynamically stable with minimal respiratory distress. Left supraclavicular and left axillary lymphadenopathy were noted. Baseline investigations were done which showed anemia, leucopenia, elevated ESR and serum LDH levels. Liver enzymes were elevated. Renal parameters were normal. Peripheral blood smear showed microcytic hypochromic anaemia. Abdominal ultrasonography showed hepato-splenomegaly. Chest X-ray revealed bilateral hilar prominence with normal lung fields. CT scan of Chest showed enlarged mediastinal lymph nodes with patchy ground glass opacities in the posterior segments of both lower lobes and mild right side pleural effusion. Sputum for Acid Fast Bacilli (AFB) was negative. Mantoux test done was negative. Lymph node biopsy of left axillary node and supraclavicular node showed non-necrotising granulomatous inflammation. GeneXpert of the biopsy sample was negative for AFB. Serum ACE level was 105

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and calcium was 11. After discussing with general physician and infectious disease consultant, provisional diagnosis of sarcoidosis was made and patient was started on steroid therapy.

Patient presented within two weeks of starting steroid therapy with complaints of progressive worsening breathlessness. On evaluation he was having tachycardia, tachypnea, hypotension requiring vasopressor support after initial fluid resuscitation. He was managed initially on non-invasive ventilation (NIV). Empirical broad spectrum antibiotics were administered followed by blood cultures. Chest X-ray showed bilateral diffuse infiltrates involving entire lung fields. Blood gas analysis revealed severe hypoxaemia with metabolic acidosis. Lab investigations showed microcytic hypochromic anaemia with neutrophilia and mild thrombocytopenia. Acute kidney injury was noted with serum creatinine of 2.3. He was intubated and mechanically ventilated due to failure of NIV trial. The patient was managed with pressure controlled ventilation for severe ARDS and broncho-alveolar lavage (BAL) sent for bacterial cultures and H1N1 PCR. BAL showed acid fast bacilli and Xpert MTB was detected with no rifampicin resistance. H1N1 PCR was negative. Screening for other common tropical infections was negative and initial blood and urine cultures showed no bacterial growth. Infectious disease consultant along with Intensivist and Nephrologist were actively involved in the management of the case. Acute kidney injury was managed with sustained low efficiency dialysis (SLED). Patient was initially started on liver sparing anti-tubercular therapy comprising of Isoniazid, Rifampicin, Moxifloxacin and Ethambutol. Patient gradually improved with dialysis and lung protective ventilation and was gradually weaned off ventilator. His liver enzyme levels returned to baseline and Pyrazinamide was added to the treatment. Acute kidney injury gradually improved and the patient was shifted to the step down intensive care unit. CT thorax done showed bilateral dense consolidation with necrotic mediastinal lymphadenopathy. Hospital course was complicated with catheter associated urinary tract infection with Klebsiella Pneumoniae which was ESBL and carbapenemase producing species and hence antibiotics were escalated to colistin. However, he did not respond well to the treatment, got progressively sick with increasing respiratory distress and shock and was re-intubated. He further developed nosocomial pneumonia and repeat endotracheal cultures grew Klebsiella Pneumoniae which was resistant to Colistin and sensitive only to Fosfomycin. He was appropriately treated with Fosfomycin, to which he did respond well. He was off vasopressors but developed critical illness polyneuromyopathy and subsequently required prolonged weaning. Tracheostomy was performed and he was gradually weaned off ventilator. Upon follow up he is clinically improving well with anti-tubercular therapy along with steroid therapy.

2. Discussion

Tuberculosis and sarcoidosis are chronic granulomatous diseases with similar clinical presentation, radiological features and histological characteristics. Tuberculosis is an infectious disease caused by Mycobacterium TB and characterised histologically by granuloma with caseous necrosis while sarcoidosis is an immunological disorder of unknown aetiology characterised by presence of non-caseating granuloma.[1] Tuberculosis is primarily treated with combination therapy with anti-tubercular drugs while sarcoidosis is managed medically with steroids and other immunosuppressive agents.[2] Distinguishing sarcoidosis from pulmonary tuberculosis can be a major challenge particularly in endemic countries like India where there is high prevalence of tuberculosis.[3]

Few isolated cases have been reported wherein both the conditions have co-existed. It has been noted to have mainly three patterns: (a) patients who have TB subsequently developing sarcoidosis, (b) patients developing concomitant TB and sarcoidosis, (c) chronic sarcoidosis developing TB later due to treatment related immune suppression.[4] Sarcoidosis can very rarely present with ARDS and only a few case reports are available all across the world.[5] Tuberculosis is rarely the primary cause of acute respiratory failure, reported incidence ranging from 1.5% to 5%.[6]

Our patient presented with clinical symptoms of high grade fever with cough for two weeks along with constitutional symptoms and lymphadenopathy. Even though his chest X-ray revealed clear lung fields but hilar prominence was noted and Tuberculosis was considered and investigated accordingly due to the fact that tuberculosis is endemic in India. Dilemma developed when there was no evidence of active tuberculosis and biopsies from both the nodes showed non-caseating granulomas. Alternative diagnosis was considered and further investigated. CT thorax showed multiple mediastinal lymphadenopathy, relatively normal lung parenchyma and mild pleural effusion. The patient also showed elevated ACE and serum calcium levels. Hence, provisional diagnosis of symptomatic stage 1 sarcoidosis was considered and steroid therapy was initiated. However, the patient presented within two weeks with ARDS, acute kidney injury (AKI) and shock. On further investigation, he was diagnosed with co-existing pulmonary tuberculosis. Therefore a high degree of clinical suspicion and a systematic approach to diagnostic testing is required to establish diagnosis of sarcoidosis and tuberculosis. Complications like AKI, disseminated intravascular coagulation (DIC), multi-organ dysfunction syndrome (MODS), and septic shock are poor prognostic factors in the intensive care. Hence multi-modality team based treatment is essential for increased survival in such cases (Figs. 1–3).

Sharma et al determined that in patients with tuberculosis; prolonged illness, absolute lymphopenia and elevated alanine aminotransferase (SGPT) are independent factors for ARDS, the latter two factors were noted in this case.[7] Fewer case reports have been published of similar co-existence of sarcoidosis and tuberculosis which posed a diagnostic dilemma.[8] This patient had a rare combination of co-existing sarcoidosis and tuberculosis and hence was started on anti-tubercular therapy along with steroids. He was managed with lung protective ventilation for ARDS and dialysis for AKI. As the patient started to recover gradually in hospital with supportive care, it was further complicated by hospital acquired infections with multi-drug resistant (MDR) gram negative organisms. Increasing number of multi-drug resistant in the intensive care units is a big health threat due to

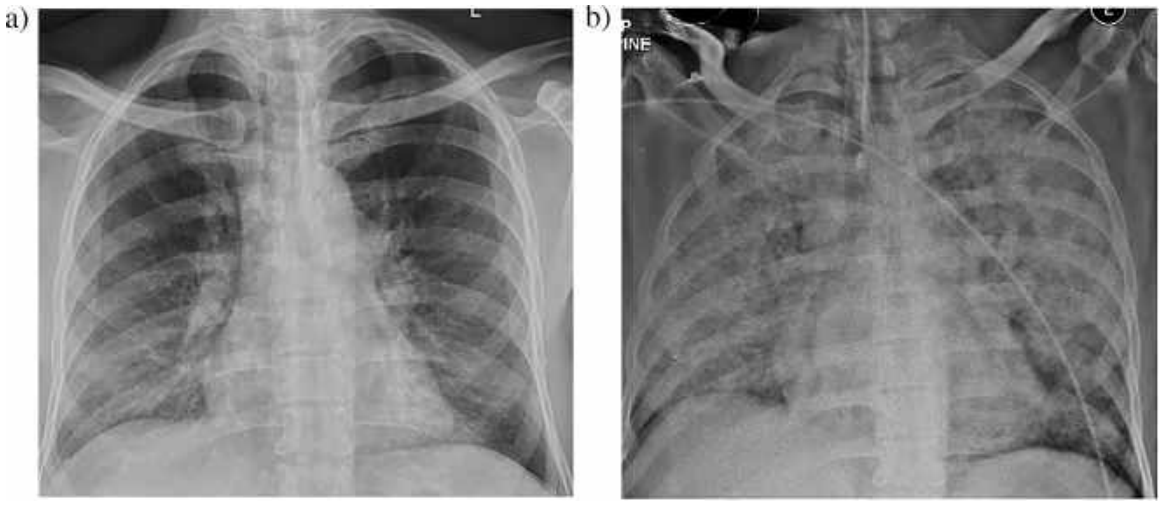

Fig. 1 – (a) Chest X-ray showing bilateral Hilar prominence (b) showing diffuse infiltrative lesions involving entire lung fields.

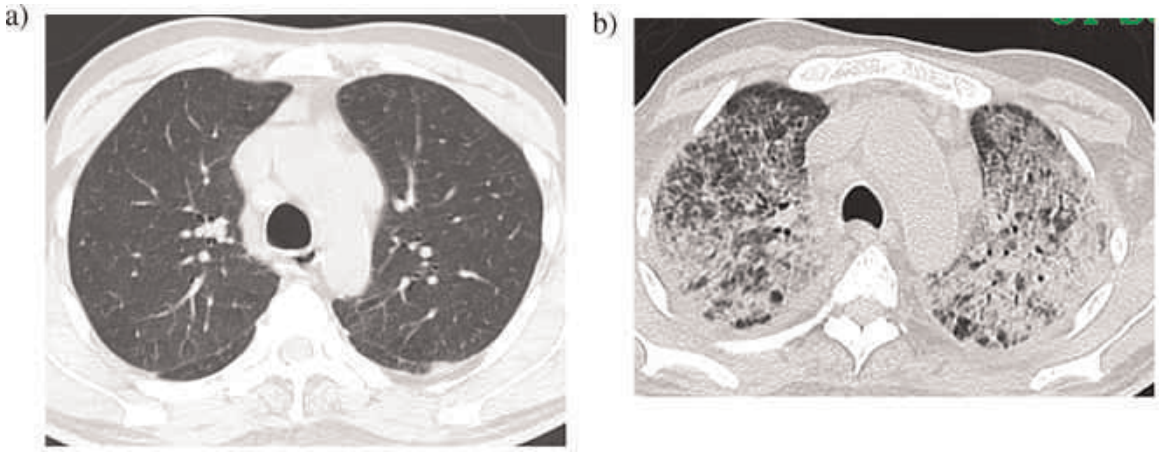

Fig. 2 – (a) Axial sections of CT thorax showing clear lung fields (b) showing dense consolidation.

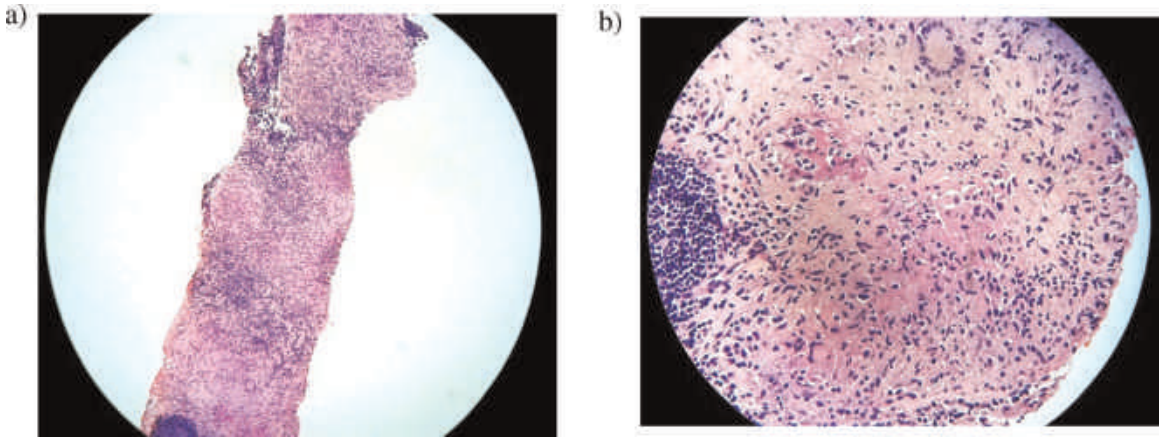

Fig. 3 – (a) Histopathology of lymph node (low power field) showing well-formed non-caseating granuloma (b) shows a non-caseating granuloma (high power field) showing presence of langhans giant cells, epitheloid cells and lymphocytes.

increased mortality and increased cost of treatment.[9] The patient gradually responded to antibiotic escalation and continued supportive care. Tracheostomy was done to facilitate weaning in this case. Patient remained to be stable on anti-tubercular therapy along with steroids.

A multi-disciplinary team comprising of Intensivist, Pulmonologist, Nephrologist and Infectious disease consultant were involved actively in the management of the above case which had several roadblocks at every step of management and which was dealt with a consensus decision in a systematic manner. To summarize, our case had a unique combination of sarcoidosis and tuberculosis with acute respiratory failure and multi-organ dysfunction which initially presented as a diagnostic dilemma and later a therapeutic challenge.

3. Conclusion

Sarcoidosis and tuberculosis diagnosis can be a major challenge in developing countries like India. There must be an increasing awareness of co-existence of both these conditions an. Multidisciplinary approach is essential not only for the diagnosis but also to help guide therapy and improve outcomes in such cases.

Conflicts of interest

The authors have none to declare.

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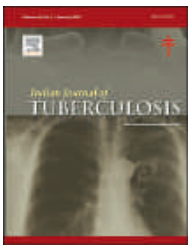

Letter to the Editor

CBNAAT is suboptimal in diagnosing tubercular pleural effusion but can be a rule in test for tubercular empyema

1. Introduction

Extrapulmonary tuberculosis (EPTB) is defined as tuberculosis of organs other than the lungs, such as pleura, lymph nodes, abdomen, etc. Diagnosis is based on one culture-positive specimen from the extrapulmonary site; or histological evidence; or strong clinical evidence consistent with active EPTB disease. In India, EPTB forms 10–15% of all types of tuberculosis.[1] Pleural TB is the second most frequent form of EPTB.[2] It is defined as excessive accumulation of fluid in pleural space, i.e. between parietal and visceral pleura. When there is accumulation of thick, purulent appearing pleural fluid in the pleural space it is called empyema.[3]

Current methods to diagnose smear negative TB and multidrug resistant TB are slow and cumbersome.[4] Smear microscopy has significant limitations because it can only be used to diagnose TB when sputum has sufficient bacillary load. It also requires skilled technician. The closest gold standard for the diagnosis of active tuberculosis is to perform culture test. However, it requires expensive and sophisticated laboratory infrastructure and staff.[5] Conventional culture techniques used for the diagnosis of drug resistant TB can take 3–8 weeks on solid media, 1–2 weeks in broth media.[4]

Nucleic acid amplifications techniques such as polymerase chain reaction (PCR) gives result much faster but still take some days and are much less sensitive and specific. They are also expensive.[6]

The cartridge based nucleic acid amplification test (CBNAAT) is a nucleic-acids amplification test for (1) the detection of *Mycobacterium tuberculosis* (MTB) complex DNA in sputum or concentrated sputum sediments; and (2) the detection of Rifampin (RIF) resistance-associated mutations of the *rpoB* gene. It integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences using real-time PCR and reverse transcriptase PCR. Because the cartridges are self-contained, cross-contamination between samples is eliminated.[7]

It is more sensitive than sputum smear microscopy in detecting TB, and it has similar accuracy as culture. Importantly, its ability to detect RIF-resistant TB in less than 2 h significantly improves the likelihood of timely treatment initiation. In December 2010, the World Health Organization (WHO) endorsed Xpert for the rapid and accurate detection of TB, particularly among HIV patients and people suspected of having multi-drug-resistant tuberculosis (MDR-TB).[5]

The scale-up of CBNAAT, or the equivalent low-touch field-friendly drug susceptibility testing (DST) option available at the time, is envisioned for the district level by 2017 to further improve and expand the capacity of the programme to interrupt transmission of drug-resistant TB.[8] The present study focuses on the utility of performing CBNAAT assay over the other investigation tools to diagnose tuberculous pleural effusions and reducing the gap time between diagnosing and starting the treatment efficiently.

2. Materials and methods

A prospective clinical study was performed under Department of Respiratory diseases in RBIPMT, Delhi from January 2014 to December 2014 in 82 sputum AFB direct smear negative suspected tuberculous pleural effusion/empyema patients without lung involvement. The enrollment were done both through out-patient department (OPD) and indoor, irrespective of age and sex.

Kingsway camp chest clinic OPD in Rajan Babu Institute for Tuberculosis and Respiratory Medicine caters approximately 8 lakhs population every year and annual new extra pulmonary case notification rate for the year 2012 is 277. As per records annual sputum negative tuberculous pleural effusion patients are approximately 80. With taking into consideration of exclusion criteria sample size was statistically estimated to be 50.

2.1. *Patient inclusion criteria*

Patient with suspected tuberculous pleural effusion and tuberculous empyema in patient aged 14 years and above.

Patient exclusion criteria:

Pleural effusion with parenchymal lesion on chest X-ray posteroanterior (PA) view.
Transudative pleural effusion.

Malignant and paramalignant pleural effusions.
Pyogenicparapneumonic effusions.

Other non-infective causes of exudative pleural effusion.

Detailed demographic and clinical data was recorded and with informed consent diagnostic pleural aspiration was performed.

Fluid aspirated sample by thoracentesis was sent for

- Direct smear microscopy for AFB bacilli.
- Cytology-TLC/differential leukocyte count (DLC)/malignant cells.
- Biochemistry for protein, sugar and LDH.
- Pyogenic culture.

If pus was aspirated by thoracentesis, it was sent for

- Direct smear microscopy for AFB bacilli.
- Pyogenic culture.

Blood aspirated was sent for protein and LDH analysis.

Fluid/pus of the patients who were suspected to be having tuberculous pleural effusion or tuberculous empyema by Light's Criteria was subjected for lymphocyte/neutrophil ratio, ADA level, AFB culture and CBNAAT. Diagnostic yield of CBNAAT was compared with respect to other conventional investigation tools and culture.

AFB culture was done at Intermediate Reference Laboratory, State TB Training & Demonstration Center, NDTB Centre, Delhi.

CBNAAT was done at Department of Microbiology, AIIMS and Department of Pathology, RBIPMT, Delhi.

2.2. Statistical analysis

The data was collected in Microsoft Excel 2010. Statistical analysis was performed using statistical package for the social science system version SPSS 17.0. Continuous variables are presented as mean ± SD. Categorical variables are expressed as absolute numbers and percentages. Association of two variables of nominal categorical data was compared using Chi-squared test or Fisher's exact test as appropriate. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) was calculated to evaluate the diagnostic accuracy of CBNAAT with culture. A *p*-value less than 0.05 was considered statistically significant.

2.3. Ethics

The study was carried out after obtaining approval from the Institutional Human Ethics Committee. An informed written consent was obtained from all the patients. Patients who showed voluntariness were enrolled in study. All patients had freedom of opting out of study at any point of time during study.

3. Results

During the study duration, 82 patients suspected of having tuberculous pleural effusion or empyema were enrolled in the study. All patients were thoroughly examined and a standard proforma was filled and a record of their history, clinical examination and diagnostic investigations were made. The patients were divided into tuberculous pleural effusion/hydropneumothorax and tuberculous empyema/pyopneumothorax. The results were tabulated *p*-value was calculated to know the significance of the results and this is implied on various parameters and compared between the test under evaluation with gold standard to know the sensitivity, specificity and positive predictive value pattern.

Out of total 82 aspirated samples there were 14 pus and 68 pleural fluid samples. Of 14 patients of empyema who were ultimately started on antitubercular treatment (diagnosed clinically/microbiologically), 12 were positive by AFB culture. Of these 12 samples, 6 were also positive for tubercular bacilli by CBNAAT (Table 1).

Out of 55 samples positive for tuberculosis by lymphocyte/neutrophil > 0.75 + ADA > 40, 23.6% (13) were diagnosed by AFB culture and 18.2% (10) were diagnosed by CBNAAT as depicted in Table 2. Results were not statistically significant.

26% (5) of samples positive by CBNAAT were also positive by AFB culture which is the true positive but 74% of sample negative by CBNAAT were positive by AFB culture (false negative). Similarly 16% (8) sample positive by CBNAAT were negative by AFB culture (false positive). 84% (41) were negative by both CBNAAT and AFB culture which is shown in Table 3. The result was not statistically significant.

Table 1 – Comparison of CBNAAT with AFB culture in pus samples.

Pus CBNAAT	Pus AFB culture				*p* value
	Positive		Negative		
	Frequency	%	Frequency	%	
Positive	6	50%	0	0%	0.473
Negative	6	50%	2	100%	
Total	12	100%	2	100%	

CBNAAT: cartridge based nucleic acid amplification test, AFB: acid fast bacillus.

Table 2 – Comparison of (lymphocyte/neutrophil + ADA) with AFB culture and CBNAAT in fluid samples.

L/N + ADA (U/L)	Fluid AFB culture			p value	Fluid CBNAAT			p value
	Pos	Neg	Total		Pos	Neg	Total	
	n	n			n	n		
L/N > 0.75 + ADA ≥ 40	13	42	55		10	45	55	
%	23.6%	76.4%	100%		18.2	81.8%	100%	
L/N ≤ 0.75 ± ADA < 40	6	7	13	0.166	3	10	13	0.707
%	46.15	53.9%	100%		23.1%	76.9%	100%	
Total	19	49	68		13	55	68	

L/N: lymphocyte/neutrophil, ADA: adenosine deaminase, AFB: acid fast bacillus, CBNAAT: cartridge based nucleic acid amplification test.

Table 3 – Comparison of CBNAAT with AFB culture in fluid samples.

	Fluid CBNAAT		Fluid AFB culture		p value
	Positive		Negative		
	Frequency	%	Frequency	%	
Positive	5	26%	8	16%	
Negative	14	74%	41	84%	0.347
Total	19	100%	49	100%	

CBNAAT: cartridge based nucleic acid amplification test, AFB: acid fast bacillus.

In our study the sensitivity of CBNAAT in pus sample is 50%, specificity is 100%, positive predictive value is 100% and negative predictive value is 74.5% and in the pleural fluid samples the sensitivity of CBNAAT is 26.3%, specificity is 83.7%, positive predictive value is 38.5% and negative predictive value is 74.5%.

4. Discussion

This study aims to evaluate role of CBNAAT in diagnosis of tuberculous pleural effusions and empyema in comparison to the gold standard AFB culture and the most commonly done test ADA and lymphocyte/neutrophil ratio. It also attempts to analyze the relation of CBNAAT positivity to different cytological and biochemical parameters so as to enable to understand the subgroup of patients where this test gives maximum yield in patients of tuberculous pleuritis and cut the long waiting time for the culture to be positive. 82 patients were enrolled in our study.

In this study 14 pus samples were tested out of which 85.7% (12) were positive for tuberculosis by AFB culture and 42.9% (6) were positive by CBNAAT. Out of 68 pleural fluid samples, 80.9% (55) were positive by lymphocyte/neutrophil ≥ 0.75 + ADA > 40 U/L, 27.9% (19) by AFB culture and 19.1% (13) were positive by CBNAAT.

Most widely used and recommended method for diagnosis of tuberculous pleural effusion is L/N > 0.75 + ADA > 40 U/L. It is one of the easier, rapid, cost effective assay and widely available.[9] As documented by Burgess et al.,[9] who have concluded that ADA when combined with differential count and lymphocyte/neutrophil ratio increases the specificity for the diagnosis of tuberculous pleuritis. In this study group 80.9% (55) cases were diagnosed by this method out of which 18.2% (10) were diagnosed by CBNAAT. It may be that for fluid samples L/N + ADA is a better tool for diagnosis than CBNAAT. Much lower yield was reported by Christopher et al.[10] in which out of 18 fluid samples positive by lymphocytic exudate with elevated ADA, 5.6% (1) was detected by CBNAAT.

On comparing CBNAAT with AFB culture the major advantage of CBNAAT is that it is a rapid test while the latter is time taking.[2] Sensitivity of both CBNAAT and AFB culture is low for diagnosis of tuberculous pleural fluid but specificity is high as suggested by Friedrich et al.[2] who reported sensitivity of Xpert was 25% while that of culture was 45% but specificity of both were 100%. Causse et al.[11] reported 11.8% (4) positive Xpert results out of 34 fluids tested all confirmed on mycobacterial culture. Hillemann et al.[12] found 2.9% (3) positive Xpert results out of 113 pleural fluid samples which were all negative on mycobacterial culture. In the study by Christopher et al.[10] out of 33 patients of tuberculous pleural effusion none were positive by AFB culture and CBNAAT. While in another study conducted by Diacon et al.[13] stated that sensitivity of pleural fluid culture was as low as 7%.

In our study sensitivity of CBNAAT in pus sample compared to the AFB culture in the study done is 50%, specificity is 100%, positive predictive value is 100% and negative predictive value is 74.5% and the sensitivity of CBNAAT in pleural fluid is 26.3%, specificity is 83.7%, positive predictive value is 38.5% and negative predictive value is 74.5%. Out of 82 patients, 17.1% (14) were diagnosed with tuberculous empyema. 42.6% (6) were positive by CBNAAT and all of these samples were also positive by AFB culture. Possible reason for higher positivity in empyema cases may be due to direct extension of the infection from thoracic lymph nodes or hematogenous spread of tuberculosis into the pleural space while pleural effusion is a hypersensitivity reaction.[14]

68 patients were diagnosed with tuberculous pleural effusion out of which 19.1% (13) were positive by CBNAAT. Out of those 13 patients, 38.5% (5) were positive for tuberculosis by AFB culture and 61.5% (8) were negative by AFB culture. 69.2% (9) fluid sample had TLC ≥ 1000 mm^3, 92.3% (12) had fluid lymphocyte > 50%, 84.6% (11) had fluid protein ≥ 3 g/dL, 69.2% (9) had sugar ≤ 60 mg/dL with fluid LDH/serum LDH ≥ 0.6 and fluid protein to serum protein ≥ 0.5 as shown in Table 4.

According to this study in cases of tuberculous empyema it was observed that AFB culture seems to have better yield in diagnosis than CBNAAT but in case of tuberculous pleural

Table 4 – Comparison of various parameters with CBNAAT in fluid samples.

Parameters in tuberculous pleural effusion cases	Present ('*n*' out of 13 CBNAAT positive cases)	%
1. Fluid TLC ≥ 1000 mm^3	9	69.2%
2. Fluid lymphocyte > 50%	12	92.3%
3. Fluid lymphocyte/neutrophil ≥ 0.75	12	92.3%
4. Fluid protein ≥ 3 g/dL	11	84.6%
5. Fluid sugar ≤ 60 mg/dL	9	69.2%
6. Fluid LDH/serum LDH ≥ 0.6	12	92.3%
7. Fluid protein to serum protein ≥ 0.5	11	84.6%
8. ADA ≥ 40 U/L	11	84.6%
9. Fluid lymphocyte/neutrophil > 0.75 + ADA > 40 U/L	10	76.9%
10. AFB culture	5	38.5%

CBNAAT: cartridge based nucleic acid amplification test, TLC: total leucocyte count, LDH: lactate dehydrogenase, ADA: adenosine deaminase, AFB: acid fast bacillus.

effusion lymphocyte/neutrophil > 0.75 + ADA > 40 U/L appears to have better yield in diagnosis followed by AFB culture than CBNAAT. In our study sensitivity of CBNAAT was low for tuberculosis pleural effusion but CBNAAT diagnosed 14.5% (8) patients out of 55 with tuberculosis supportive by clinical manifestations and other cytological and biochemical markers which were negative by AFB culture.

Thus CBNAAT is suboptimal for diagnosis of tuberculous pleural effusion, however further studies with large subgroup of TB pleuritis cases is required to study factors which can increase yield of CBNAAT in specific subgroups of patients.

5. Conclusion

CBNAAT is a rapid diagnostic test requiring fewer skills to perform the test with simultaneously detecting rifampin resistance.

This study showed that in case of diagnosis of tuberculous empyema AFB culture is superior to CBNAAT with latter having low sensitivity but 100% specificity and PPV. Thus it may be rule in test for tuberculous empyema patients but not rule out test.

In cases of tuberculous effusion fluid lymphocyte/neutrophil > 0.75 + ADA > 40 U/L showed better results than AFB culture followed by CBNAAT.

Even though the sensitivity of CBNAAT is low it diagnosed 8 cases of pleural effusion with tuberculosis where the AFB culture was negative.

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