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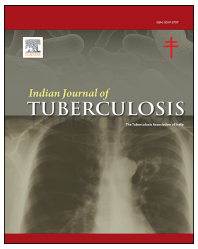

Editorial

Geriatric TB: Needs focussed attention under RNTCP

To fulfil the dream to achieve "TB Elimination by 2025" in India, all age groups of patients need to be covered under RTNCP for early diagnosis and adequate and standard anti TB treatment. The geriatric population is a bit difficult to deal with because of certain peculiar biological and behaviour changes in such patients.

Pulmonary tuberculosis (TB) in geriatric age group may cause no or mild signs and symptoms in contrast to the prolonged disease course that is common in post-primary or adult type disease. Atypical clinical manifestations of TB in older persons can result in delay in diagnosis and initiation of treatment; higher rates of morbidity and mortality from this treatable infection can occur. Underlying illnesses, age-related diminution in immune function, the increased frequency of adverse drug reactions, and institutionalization can complicate the overall outcome in elderly patients with TB. A high index of suspicion for TB in this vulnerable population is, thus, undoubtedly justifiable.[1] Acute or chronic diseases, malnutrition, and the biological changes associated with aging can disrupt protective barriers, impair microbial clearance mechanisms, and contribute to the expected age-related diminution in cellular immune responses to Mycobacterium TB.[2] The diagnosis of TB can be difficult, and this treatable infection is sometimes documented only on post-mortem examination. In addition, therapy for TB in elderly individuals is challenging because of the increased incidence of adverse drug reactions. Furthermore, institutionalized elderly persons are at high risk for reactivation of latent TB and are susceptible to new TB infection.[2]

From India, not much data on the problem of tuberculosis in the elderly are available. With changing demography of the population, and increase in the number of elderly, more and more older individuals are being diagnosed as suffering from tuberculosis. However, the problem of geriatric tuberculosis has not received the attention it deserves. It is evident from the paucity of literature on this common problem affecting the elderly. An isolated study on the profile of disease in the elderly from Himachal Pradesh has been reported.[3] However, some of the observations that have been reported in the studies of geriatric tuberculosis from the West and South-East Asian countries could very well be applicable in our setting.

Tuberculosis is the prototype of a disease in which cell-mediated immunity plays an important part in controlling the infection. It is well known that age related decline in the cell-mediated immunity influences reactivation of latent infection in the elderly. However, studies conducted on the immunoglobulin status in the geriatric pulmonary tuberculosis patients, have shown no deficiency in their humoral responses.[4] It has been observed that the cytokine production in response to stimulation with *Mycobacterium tuberculosis* is well preserved in old age.[5] In individual cases, presence of intercurrent illnesses like diabetes mellitus, chronic renal failure, malnutrition, alcohol abuse, certain malignancies and use of immunosuppressive drugs like corticosteroids further impair cell mediated immunity. These can thereby increase the risk of reactivation of the disease. Adverse social factors and poor living conditions also affect the elderly much more than the young.

Tuberculosis in older patients can present atypically.[6,7] Approximately 75% of elderly persons with tuberculosis disease manifest lung involvement.[8] In addition, disseminated or miliary tuberculosis, tuberculous meningitis, and skeletal and genitourinary tuberculosis increase in frequency with advancing age.[9] Many older patients with tuberculosis disease may not exhibit the classic features of tuberculosis (i.e., cough, hemoptysis, fever, night sweats, and weight loss). Tuberculosis in this population may present clinically with changes in functional capacity (e.g., activities of daily living), chronic fatigue, cognitive impairment, anorexia, or unexplained low-grade fever.[6,7] Nonspecific symptoms and signs that range in severity from subacute to chronic and that persist for a period of weeks to months must alert clinicians to the possibility that unrecognized tuberculosis is present.

Sputum examination for *M. tuberculosis*, using smear and culture, is indicated for all patients who have pulmonary symptoms and/or radiographic changes compatible with tuberculosis and who have not been treated with tuberculosis chemotherapy. More aggressive diagnostic intervention should be considered for elderly patients who are unable to expectorate sputum; the use of flexible fiberoptic bronchoscopy to obtain bronchial washings and bronchial biopsy specimens is clearly feasible and is a valuable diagnostic option.[10] In frail elderly patients, however, the risk of such a procedure should be carefully weighed against the benefit of potentially making a diagnosis of tuberculosis.

For suspected pulmonary tuberculosis, it is recommended that 2 sputum specimens; one obtained in the morning and one spot be used for routine mycobacteriological studies as per RNTCP guidelines. These specimens should be subjected to smear examination and then cultured for *M. tuberculosis*.

Rapid molecular tests based on nucleic acid amplification tests, such as CBNAAT and LPA for amplifying DNA may facilitate rapid detection of *M. tuberculosis* in respiratory tract specimens. The rapid diagnosis of tuberculosis is especially important in the high-risk elderly population and for HIV-infected persons and

patients infected with multiple-drug–resistant *M. tuberculosis* (MDR-TB). Histologic examination of tissue from various sites, such as the liver, lymph nodes, bone marrow, pleura, and synovium, that reveals the characteristic tissue reaction (caseous necrosis with granuloma formation) is also useful for diagnosis of tuberculosis disease.

Old people with tuberculosis present problems not only of the diagnosis but also of treatment. The key problems are a poor compliance with treatment, poor tolerance of therapy and the presence of underlying or associated diseases.[11] The main cause of failure of treatment in tuberculosis, whatever the age, is poor patient compliance, and in the elderly this problem is accentuated. Old people especially the very old are unreliable about taking tablets regularly, at the right time or in the right dose, particularly if several drugs are to be taken concurrently. Poor memory, poor eyesight and mental confusion may be contributory factors. Old people often become apathetic about their treatment and lack the determination required to complete a course of treatment of six months. Many countries, therefore, prefer to use supervised intermittent chemotherapy for such patients. Side effects of certain drugs may also lead to poor compliance with the treatment. A careful watch must be kept for the side effects of drug treatment because the old persons, particularly the very old, cannot be relied upon to recognize their significance. Doses of drugs must be carefully monitored and special care taken if there is evidence of hepatic or renal failure. In a retrospective review, it has been reported that elderly people were nearly three times more likely to have reactions to antituberculous drugs as compared to younger patients.[12] Various studies including those from India have definitely shown advancing age as an important predictor of hepatotoxicity due to INH and rifampicin.[12,13] Rifampicin combined with INH has an additive but not synergistic hepatotoxic effect. Monthly monitoring of serum transaminases is advisable in such patients.

Ethambutol can cause diminution of visual acuity, central scotomas and disturbance of red–green vision attributable to optic neuritis. Since some visual impairment is common in elderly, a careful examination that includes testing of visual acuity and color discrimination should be performed before initiating ethambutol therapy. In elderly patients with significant renal dysfunction associated with retinopathy, or cataracts, in whom evaluation of visual changes may be difficult, the benefits of ethambutol administration must be carefully weighed against the risks. The nephrotoxicity and ototoxicity due to streptomycin is more frequent in patients with preexisting renal impairment and is generally irreversible. Since with normal aging, renal function declines, hearing acuity diminishes, and vestibular disturbances are more incapacitating, elderly patients have increased risks of suffering from renal toxicity and ototoxicity and of being severely impaired by them. As with ethambutol, the benefits of streptomycin therapy must be weighed against its risks in the elderly patient and dose adjusted accordingly.

Tuberculosis in geriatric population is not so uncommon. It is a serious illness in this age group. Elderly people are at a high risk of developing disease and sometimes disseminated type also. Clinical presentation is usually atypical sometimes clinical features are masked by other co-existing disease. Diagnosis is difficult as tuberculin test is negative because of age related anergy; radiological features are also atypical as COPD usually coexists. As far as treatment is concerned, dosage of drugs have to be adjusted, more chances of side effects (due to some or other concomitant therapy), difficult compliance. But if the care givers of old persons are alert, early diagnosis and ensuring treatment adherence do help in managing TB cases in this age group.

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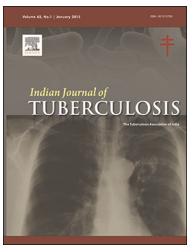

Original article

Role of line probe assay in detection of extra-pulmonary tuberculosis: Experience from a tertiary care hospital in western Maharashtra

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ABSTRACT

Background: Diagnosis of extra pulmonary tuberculosis (EPTB) is challenging due to its atypical clinical presentation and frequently results in a delay or deprivation of treatment. Apart from rapid case detection, early determination of MDR status is imperative in such situations. The commercially available Geno Type MTBDRplus assay version 2.0 (Hain Lifescience, Nehren, Germany) detects both the presence of *Mycobacterium tuberculosis* (MTB) complex as well as the presence of INH and Rifampcin resistance. We aim to evaluate the role of this test in diagnosis and detection of resistance by comparing its performance against gold standard i.e. culture and against the composite reference standards (CRS) in the diagnosis of EPTB.

Material and methods: The data of 130 EPTB samples processed form January 2014 till May 2017 at Poona Hospital and Research centre were selected for the study. All the samples were processed for Ziehl–Neelsen stain, Geno Type MTBDRplus assay (LPA) and liquid automated culture (BacT/Alert) simultaneously. Geno Type MTBDRplus assay (LPA) was performed directly on the samples. The 24 samples giving positive results on LPA and grown *M. tuberculosis* on culture were subjected to anti mycobacterial susceptibility testing for 1st line anti-tubercular drugs by BACTEC MGIT 320 system.

Results: Out of 130 samples, 7 samples grew atypical mycobacterium and all the 7 samples turned negative on Line Probe Assay. Direct LPA on processed samples yielded 48/130 (36.9%) positivity. Geno Type MTBDRplus assay was positive for *M. tuberculosis* in (72.09%) 31/43 culture positive cases and (21%) 17/80 of culture negative cases. Geno Type MTBDRplus assay sensitivity and specificity results were assessed in comparison to CRS made up of culture results and clinical, radiological and histological findings. The overall sensitivity of Geno Type MTBDRplus assay was 45.19% (47/104) and specificity was 94.73 (18/19). Out of 24 samples which were compared for results between LPA and culture, Geno Type MTBDRplus assay accurately identified 3 of 3 of Rifampcin resistant strains and 20 of 21 Rifampcin

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sensitive strains. Geno Type MTBDRplus assay identified 4 of 4 INH resistant strains and 19 of 20 INH sensitive strains and MDR was obtained for 3 of 3 strains.

Conclusions: Geno Type MTBDRplus assay can give early diagnosis and sensitivity for both INH and Rifampcin in extra pulmonary samples. More number of studies is further required to establish Geno Type MTBDRplus assay as an important tool for obtaining diagnosis and resistance to first line drugs in extra pulmonary samples.

1. Introduction

TB is a multisystem disease with myriad presentations and manifestations; it can affect any organ or tissue. The public health emphasis however is on infectious pulmonary TB. Nevertheless, extra-pulmonary tuberculosis (EPTB) remains extremely common and is probably under recognized and inappropriately treated condition.[1] EPTB takes many forms, and evidence regarding best practice for many aspects of case finding, diagnosis and treatment is lacking.[1] The burden of EPTB is high, ranging from 15% to 20% of all TB cases in HIV-negative patients, while in HIV-positive people it accounts for 40–50% of new TB cases.[2] EPTB refers to TB involving organs other than the lungs (e.g., pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, or meninges).

In India, 10–15% of TB cases are estimated to be cases of EPTB (which affects mainly the lymph nodes, meninges, kidney, spine, and growing ends of the Bones), with a 25–50% case mortality rate within months.[3] In this situation, not only rapid TB case detection but also the early determination of MDR status is important. The major challenge in the diagnosis of EPTB is the frequently atypical clinical presentation simulating other inflammatory and neoplastic conditions, which frequently results in a delay in diagnosis and consequently therapy.[4]

WHO and Indian guidelines have endorsed rapid molecular testing with Gene Xpert for extra pulmonary specimens.[5]

However, the test does not detect resistance to Isoniazid (INH) which is the most common form of mono resistance with a prevalence of 10% among new tuberculosis (TB) cases and 28% among retreatment cases reported globally.[6] Despite high prevalence, detection of INH-resistance has received lower priority, largely because the clinical impact of INH-monoresistance is less pronounced. The extent of treatment failure, recurrence, and acquisition of further resistance development in patients with INH-monoresistance remains an issue of debate; however meta-analysis suggests higher rates of failure or relapse and acquired resistance.[7]

The commercially available Geno Type MTBDRplus assay version 2.0 (Hain Lifescience, Nehren, Germany) detects both the presence of MTB complex as well as the presence of INH and RIF resistance. However, data is lacking regarding its role in diagnosis of EPTB especially EP MDR TB.

This study is conducted in a private, super specialty tertiary care hospital, receiving samples from Pune city and adjoining regions of western Maharashtra. The current study was a retrospective study to assess the utility of performing Geno Type MTBDRplus assay (LPA) directly on patient's samples for the diagnosis of EPTB in comparison with a gold standard test i.e. culture. We have also combined the results of different reference test and prepared our composite reference standards (CRS) to evaluate the true diagnostic potential of Geno Type MTBDRplus assay (LPA). The CRS for this study was composed of liquid culture method, clinical findings, histology/cytology/Zn smear, site specific USG, computerized tomography scan/magnetic resonance imaging.[4]

2. Materials and methods

Study population and samples: The study was performed retrospectively on extra pulmonary samples received at private tertiary care hospital, Pune, India from January 2014 till May 2017. Direct Geno Type MTBDRplus assay (LPA) and liquid culture was simultaneously performed only on 130 samples during this period. Only these samples were included in study and their clinical history, Radiology, Histology/cytological records were reviewed.

Methods

i) Extra pulmonary samples received in the laboratory like pus, aspirates and fluids were processed directly while Lymph nodes and tissues were minced properly and caseaous portion was subjected to Ziehl–Neelsen (ZN stain) staining on all extra pulmonary samples. The processing of samples for ZN staining was done as per the guidelines of RNTCP. ZN staining and Geno Type MTBDRplus assay (LPA) was performed on all 130 samples directly and all samples were cultured simultaneously.

ii) The samples were further digested and decontaminated by NALC-NaOH method. DNA was extracted from the part of decontaminated samples using Genolyse® kit (Hain Lifescience GmbH, Nehren, Germany) as per manufacturer's instructions.[8] The extracted DNA was processed by the LPA using GenoType®MTBDRplus (Hain Lifescience GmbH, Nehren, Germany) for detection of MTB complex and RIF and/or INH resistance. A positive *Mycobacterium tuberculosis control (TUB) band* indicated the presence of members of the *M. tuberculosis complex*. The run was considered valid if the negative controls showed the presence of conjugate control and amplification control bands only. Incomplete amplification of RIF and/or INH genes or absence of TUB with an evaluable resistance pattern was considered as an invalid result and the test samples were repeated.

iii) Liquid cultures were performed on remaining decontaminated samples using automated BacT/Alert MP (Biomeriux) system as per manufacturer recommendations.[9]

BacT/Alert system is a colorimetric fully automated non-radiometric liquid culture system. Briefly the digested decontaminated samples were added in BacT/Alert MP bottles along with MB/BacT/Alert Antibiotic Supplement and incubated in BacT/Alert system at 37 degrees for 6 weeks. On receipt of positive signal from machine, the bottle was removed, ZN smears prepared and observed for presence of mycobacterium. Isolates showing rapid growth or atypical morphology were subjected to immunochromatographic (SD test)[10] to rule out atypical mycobacterium.

iv) The positive cultures for *M. tuberculosis* were subjected to anti mycobacterial susceptibility testing for 1st line anti tubercular drugs namely INH, Rifampcin, Ethambutol, Pyrizinamide and Streptomycin at a concentration of 0.1 μg/ml, 1 μg/ml, 5 μg/ml, 100 μg/ml, and 1 μg/ml respectively by BACTEC MGIT 320 system. The sensitivity was carried out at Golwilkar Metropolis Laboratory, Pune.

v) Patient categories: based on the CRS,[4] patients were categorized into 4 groups:
Confirmed TB cases: culture positive cases including smear positive and negative status
Probable TB cases: culture negative, but showing clinical symptoms, radiological findings, and/or histology/cytology suggestive of TB
Possible TB cases: only clinical symptoms and signs suggestive of TB
Not TB cases: culture and all other tests for TB were negative; patient was not administered treatment for TB, other definitive diagnosis obtained.

Table 1 shows the signs and symptoms taken into consideration according to the site of infection from where the specimen was obtained.

3. Results

The majority of patients were from the age group 20–40 yrs 66/130 (50.7%), followed by 40–60 yrs 33/130 (25.3%).

Among 130 cases, 74 were males and 56 were females giving with a male to female ratio of 1.3:1.

The breakup of the 130 EPTB samples is shown in Table 2. The commonest sample received was pleural biopsy (32.3%) followed by pus (23.8%) and lymph node (18.4%) samples. Out of 130 samples, 52 (40% positivity) samples were positive for ZN stain, 48 (36.9%) were positive for LPA – MTBDRplus and 43 (33.07%) by culture method as detailed in Table 2.

Out of 130 samples, 7 samples grew atypical mycobacterium and all the 7 samples were negative on Line Probe Assay. These patients were excluded from further study.

Among 123 patients, 43/123 (34.95%) were culture positive confirmed cases: 33/123 (26.82%) being smear positive and 10/123 (8.1%) being smear negative. Clinically, radiologically, and/or histologically/cytologically positive case were 56/123 (45.52%), suggestive of probable cases: 5/123 (4.06%) were only clinically positive possible cases: and 19/123 (15.44%) patients had culture and all other tests for TB negative, no ATT was given to them, other definitive diagnosis obtained. All CRS positive patients comprised of 104 cases.

Table 1 – Signs and symptoms taken into consideration based on site of infection.

System	Symptoms
Lymph nodes	Enlargement of lymph node, mass formation in the neck
Cardio respiratory	Shortness of breath, hypertension, chest pain, dyspnea
Brain	Irritability, restlessness, neck stiffness, headache persistent for 2–3 weeks, vomiting, seizures, change in metal condition or behavior
Intestinal tract, abdomen	Abdominal pain, diarrhea
Spinal	Inability to walk, move hands, numbness, weakness in limbs, pain and swelling at the site, paraspinal muscle spasm
Weight loss, persistent cough and fever for 2–3 weeks were common symptoms for all kinds of specimen.	

Table 2 – Types of extra pulmonary samples and positivity on different samples.

Sample	Number	ZN microscopy positive	MTBDRplus direct positives	BacT/Alert positives
Pus	31 (23.8%)	18/31	19/31	12+ (1 atypical)/31
LN	24 (18.4%)	19/24	15/24	14+ (4 atypical)/24
PL Biopsy	42 (32.3%)	10/42	8/42	10+ (2 atypical)/42
CSF	2 (1.5%)	0/2	1/2	1/2
PL FL	8 (6.15%)	0/8	1/8	1/8
TBNA	12 (9.2%)	2/12	2/12	3/12
Tissue	6 (4.6%)	2/6	1/6	1/6
FNA	3 (2.3%)	0/3	0/3	0/3
Miscellaneous	2 (1.5%)	1/2	1/2	1/2
Total	130	52/130 (40%)	48/130 (36.9%)	43/130 (33.07%) + 7 atypical

ZN microscopy: Ziehl–Neelsen microscopy; LPA: line probe assay; PL FL: pleural fluid; BacT/Alert: BacT/Alert MP culture (BioMeriux); PL Biopsy: pleural biopsy; TBNA: transbronchial needle aspiration; FNA: fine needle aspiration.

Out of 123 patients, 13/123 (10.56%) patients were HIV positive.

Geno Type MTBDRplus in comparison to culture showed a sensitivity of 72.09% (31/43) and specificity of 78.75% (63/80) as per Table 3.

Upon comparison with a CRS, the overall sensitivity of culture was found to be 41.43% (43/104).The sensitivity and specificity of MTBDRplus against the CRS was found to be 45.19% (47/104) and 94.73% (18/19) respectively. MTBDRplus showed sensitivity of 76% (19/25) and 75% (15/20) in case of pus and lymph node samples when compared with CRS (Table 4).

Among Confirmed TB cases, LPA showed sensitivity of 81.81% (27/33) in smear positive cases and 60% (6/10) in smear negative cases. In Probable TB patients, LPA had sensitivity of 78.57% (11/14) in smear positive cases and 7.14% (3/42) in smear negative cases.

17 of the 123 patients were already receiving ATT for more than 2 months. 6 of them gave valid results on LPA, while only 1 sample showed growth in culture.

For assessing the performance of Geno Type MTBDRplus assay, MGIT 320 automated culture system was taken as a gold standard. Out of 123 samples, valid LPA results and sensitivity by MGIT 320 automated culture system was obtained in 24 cases. Geno Type MTBDRplus assay accurately identified 3 of 3 of Rifampcin resistant strains and 20 of 21 Rifampcin sensitive strains. Geno Type MTBDRplus assay identified 4 of 4 INH resistant strains and 19 of 20 INH sensitive strains and MDR was obtained for 3 of 3 strains. 1 sample which was detected MDR by Geno Type MTBDRplus assay turned out to be sensitive to Rifampcin and INH by culture method. The sensitivity and specificity for detection of Rifampcin was 95.23% and 100% respectively and for INH was 95% and 100% respectively (Table 5).

4. Discussion

In the present study we attempted to find out whether performing line probe assay by Geno Type MTBDRplus assay directly on extra pulmonary samples routinely will benefit the patients by providing reliable results in a very low turnaround time.

In the present study, most of the clinically suspected patients were between age group 20–40 yrs with a male to female ratio of 1.3:1 which correlates with studies of Rama Lakshmi et al.[11] (patients were between the age groups of 21

Table 3 – Comparison between MTBDRplus assay and MTB BacT/Alert MP culture.

Genotype	MTB BacT/Alert MP culture			
MTBDRplus	Positive	Negative	Total	
Positive	31	17	48	Sensitivity = 72.09%
Negative	12	63	75	Specificity = 78.75%
	43	80	123	ppv = 64% npv = 84%

Table 4 – Sensitivity and specificity of culture and LPA with respect to different specimen group in comparison with CRS.

Method compared to CRS	Pus	Lymph node	Pleural biopsy	Body fluids	Aspirations (TBNA, FNA)	Tissue and biopsy	Miscellaneous	Total (pooled)
	n = 30	*n* = 20	*n* = 40	*n* = 11	*n* = 15	*n* = 6	*n* = 1	*n* = 123
Culture sensitivity	48% (12/25)	70% (14/20)	32.25% (10/31)	30% (3/10)	25% (3/12)	6.25% (1/6)		41.43% (43/104)
Geno Type MTBDRplus (LPA) sensitivity	76% (19/25)	75% (15/20)	25.8% (8/31)	30% (3/10)	16.66% (2/12)	0% (0/6)		45.19% (47/104)
Geno Type MTBDRplus (LPA) specificity	100% (5/5)	–	88.89% (8/9)	100% (1/1)	100% (3/3)	–	100% (1/1)	94.73% (18/19)

CRS: composite reference standards; TBNA: transbronchial needle aspiration; FNA: fine needle aspirations.

Table 5 – Concordance between Geno Type MTBDRplus assay and MGIT 320 automated culture system.

Genotype	MTB BacT/Alert MP culture				
MTBDRplus	Rifampcin		Isoniazid		
	s	r	s	r	
rif s	20	0			Sensitivity = 20/21 = 95.23%
rif r	1	3			Specificity = 3/3 = 100%
inh s			19	0	Sensitivity = 19/20 = 95%
inh r			1	4	Specificity = 4/4 = 100%

s: sensitive; r: resistant.

and 40 yrs, with male: female ratio of 1.8:1) and Siddiqui et al.[12] with a male to female ratio of 2.03:1.

The comparatively high rates of positivity by Liquid culture method in case of Lymph node and Pleural biopsy samples is due to growth of atypical mycobacteria in culture, for which Geno Type MTBDRplus gave negative results.

The positivity of liquid culture for detection of AFB by automation is reported to be higher (30%)[13,14] and earlier than culture on LJ Medium. We found it to be around 33.07% in our study.

Direct LPA on processed samples has shown lower sensitivity of detection (45.19%) when compared with CRS and it is only slightly higher than sensitivity of culture 41.43%. However, the sensitivity of detection was high 76% and 75% in case of pus and lymph nodes respectively. The sensitivity of direct LPA on pleural biopsy is lower (25.85%) than sensitivity of culture (32.25%). Sanker et al. have reported positivity of 58.3% for lymph nodes, 49% for aspirated pus and 33.3% for solid tissues. Poor positivity is reported for body fluid like CSF, pleural fluid and peritoneal fluid and other solid tissues which is similar to our study.[15]

The other molecular tests like Gen Xpert have shown higher sensitivity 83.7% and specificity of 99.2% in samples like lymph node tissue and aspirates on comparison with CRS.[5]

In our study, MTBDRplus has given sensitivity of both INH and Rifampcin in a small group 11 of 14 patients (78.57%) of culture negative smear positive Probable TB cases. The test has also detected resistance pattern in 6 out of 17 (35.2%) patients already receiving ATT, while culture detected only 1 case in 17 samples (5.8%). Study should be conducted on larger sample size to know whether the test will be useful in patients already receiving ATT and on the smear positive extra pulmonary samples.

Overall concordance of Geno Type MTBDRplus assay with conventional DST was 94.11% in a study performed by Kumari et al.[16] However, the study was performed on DNA extracted from MTB culture and not on direct samples. In our study, on comparison between Geno Type MTBDRplus assay with conventional liquid culture DST, the sensitivity and specificity for detection of resistance was high for both INH and Rifampcin. This should be further confirmed by conducting the test on more number of samples so the patients of EPTB will get the treatment at the earliest without missing INH resistance status.

In summary, Geno Type MTBDRplus assay could rapidly identify Rifampcin and INH sensitivity and resistance within two to four days in good number of patients by direct testing of processed clinical specimens.

To the best of our knowledge this is one of the few studies providing data on performance of direct LPA in EPTB patients in India and adds to limited evidence available globally.

5. Conclusions

As early diagnosis of EPTB is challenging because of the paucibacillary nature of the infections, procedures such as NAATS with enhanced sensitivity is required and to be available for diagnosis of EPTB. Geno Type MTBDRplus assay can give early diagnosis and sensitivity for both INH and Rifampcin in extra pulmonary samples. More number of studies is further required to establish Geno Type MTBDRplus assay as an important tool for obtaining diagnosis and resistance to first line drugs in extra pulmonary samples.

Conflicts of interest

The authors have none to declare.

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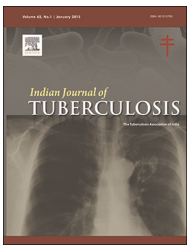

Original article

Epidemiological and behavioural correlates of drug-resistant tuberculosis in a Tertiary Care Centre, Delhi, India

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ABSTRACT

Background: Multidrug-resistant tuberculosis (MDR-TB) is a major public health challenge in India. It is associated with poor treatment outcomes, multiple adverse effects to treatment and involves enormous social and economic losses. The objective of the study was to ascertain the epidemiological and behavioural correlates contributing to drug resistance among patients admitted in a tertiary hospital in Delhi with drug-resistant TB (DR-TB).

Methodology: A descriptive cross-sectional study was carried out during the period of July–November 2013 at the Rajan Babu Institute of Pulmonary Medicine and Tuberculosis 7 (RBIPMT), Delhi. All patients admitted with DR-TB for treatment were interviewed regarding social, demographic, and treatment aspects, using a semi-structured questionnaire. Their medical records were also reviewed.

Results: A total of 250 patients were included in the study; 198 (79.2%) with multidrug-resistant (MDR-TB) and 52 (20.8%) with extensively drug-resistant TB (XDR-TB). Of these, 66% patients were male and 46% came from poor socioeconomic background. All the patients had history of receiving anti-tubercular treatment (a mean of 2.3 times, range 1–6 times) before the current diagnosis of DR-TB. While 81 (32%) took treatment from private practitioner during the first episode of TB, 146 (58%) received treatment exclusively at government health facilities. Almost 87% of DR-TB patients were previously treated with category-II under RNTCP. Irregularity of treatment was reported by 88 (35%) patients.

Conclusion: The study explores the epidemiological and behavioural correlates among the patients with drug-resistant TB. History of previous treatments for TB was a common feature among all the enrolled patients. The fact that more than half of DR-TB patients received anti-tubercular treatment exclusively in government facilities is a matter of concern. There is an urgent need to ensure treatment adherence through improved quality in service delivery in public sector and strong linkage with the private sector. Health education and patient counseling is needed to address personal level risk factors and to ensure treatment adherence.

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1. Introduction

Multidrug-resistant tuberculosis (MDR-TB) is a global public health challenge of 21st century. *Mycobacterium Tuberculosis* (TB) may develop resistance to the anti-tubercular drugs. This can manifest as multidrug-resistant TB (MDR-TB), in which the bacilli is resistant to isoniazid and rifampicin with or without resistance to other drugs, or extensively drug-resistant TB (XDR-TB) in which the bacilli is resistant to at least isoniazid, rifampicin, a fluoroquinolone and a second-line injectable anti-TB drug.[1] According to the World Health Organization (WHO), globally in 2016, an estimated 4.1% of new cases and 19% of previously treated cases had MDR-TB.[2] In 2016, there were 4,90,000 new cases of MDR-TB with India, China and the Russian federation accounting for nearly half (47%) of the global burden of MDR-TB.[2] The management of drug resistant TB requires early diagnosis with prolonged treatment which poses a higher risk of adverse drug reactions, escalation of treatment costs and lower chances of cure. Only 46% of MDR-TB patients in India who were initiated on treatment were reported to have successful treatment outcomes in 2016.[3] India has a high burden of TB and to control it the government of India launched the Revised National Tuberculosis Control Programme (RNTCP) with Direct Observed Treatment Short-course (DOTS) treatment scheme in the year 1997 with the twin objectives of achieving and maintaining a TB treatment success rate of at least 85% among new sputum positive (NSP) patients and to achieve and maintain detection of at least 70% of the estimated new sputum positive people in the community which were achieved since 2007.[4–6] The programme provides free services for detection and treatment through a network of district TB centers (DTC), designated microscopic centres and DOTS centres. An estimated 7.75 million lives have been saved by India's RNTCP from 1997 to 2016 by averting transmission and improving treatment outcomes in TB patients.[7]

However, the emergence of MDR-TB and XDR-TB threatens the remarkable decadal gains made in control of the disease due to high morbidity and mortality among the younger, economically productive age-groups and spread of drug-resistant strains of TB into communities.[8–10] It has been predicted based on modeling estimates that in absence of improvement in TB management practices over the next 2 decades, India would witness a gradual transformation of the current epidemic of drug-susceptible TB to a drug-resistant epidemic by 2032.[11]

Hence, it is essential to understand the epidemiological correlates operating both at patient level and health system level that are associated with potential generation of DR-TB in a resource constrained setting to generate evidence base for enacting informed policy decisions for control of drug-resistant TB.

The study was conducted with the objective of ascertaining the epidemiological correlates – sociodemographic, environmental, personal and treatment related among drug-resistant (MDR and XDR) TB patients.

2. Methods

We conducted a descriptive cross-sectional study at the Rajan Babu Institute of Pulmonary Medicine and TB (RBIPMT), situated in North Delhi. RBIPMT is the largest institute providing medical care in TB and pulmonary diseases in Asia equipped with 1600 beds for TB, MDR-TB and XDR-TB patients. The institute serves as the main tertiary care centre for the management of TB and the drug resistant TB in the Delhi city.

We enrolled for study all patients diagnosed as MDR-TB or XDR-TB who were admitted at this hospital for initiation of treatment. We obtained written and informed consent in adults and assent in minors from all the patients prior to their enrolment. We excluded patients who were seriously ill like those on ventilator and those incapables of comprehending and responding to the interviewer from the study.

We used the operational definitions of drug-resistant TB as per the RNTCP guidelines.[1] For example, an MDR-TB case was defined as a TB patient whose sputum was culture positive for *Mycobacterium tuberculosis* and is resistant in-vitro to isoniazid and rifampicin with or without other anti-tubercular drugs based on drug-susceptibility testing (DST) results from an RNTCP-certified Culture and DST Laboratory. XDR-TB case was defined as an MDR-TB case whose recovered *M. tuberculosis* isolate was resistant to at least isoniazid, rifampicin, a fluoroquinolone (ofloxacin, levofloxacin, or moxifloxacin) and a second-line injectable anti-TB drug (kanamycin, amikacin, or capreomycin) at a RNTCP-certified Culture and DST Laboratory. The diagnosis of drug-resistant TB in the patients was made at the New Delhi TB Centre (NDTB), the designated Intermediate Reference Laboratory (IRL) under RNTCP for the state of Delhi. The functions of IRL include supervision and monitoring of EQA activities, providing Mycobacterial Culture and DST services and training. The strategy for diagnosis of drug resistant TB at NDTB employed a solid egg-based Lowenstein–Jensen (LJ) media for culture. DST was conducted for both first and second line drugs while Line Probe Assay (LPA) was used for rapid diagnosis of the patients.

The treatment status of the patient was assessed using RNTCP guidelines. A relapse was defined as a TB patient who was declared cured or treatment completed by a physician, but who reports back to the health service and is found to be sputum smear positive. A defaulter was defined as a patient who received anti-TB treatment for one month or more from any source and who returned to treatment after not taking anti-TB drugs consecutively for duration of 2 months or more. A treatment failure was defined as a smear positive patient who was still smear positive 5 months after initiation of treatment, or a patient who was initially smear negative but who becomes smear positive during the course of treatment. Irregularity of treatment was defined as patient who deviated or discontinued the recommended anti-tubercular treatment during any of his/her previous TB treatment episodes.

For the purpose of the study, overcrowding was considered if floor space area per person was less than 50 square feet or if 2 persons not husband and wife, of opposite sex were obliged to sleep in the same room.[12] Ventilation status was assessed

Table 1 – Socio-demographic profile of study participants (N = 250).

Variable	Number (n = 250)	Percentage	(95% CI)
Median age in years (range)	26 (14–67)		
Gender			
Male	165	66	(59.7–71.8)
Female	85	34	(28.2–40.2)
Education level			
Illiterate	47	18.8	(14.2–24.2)
Literate below high school	70	28	(22.7–37.3)
High school pass and higher	133	53.2	(47.1–59.7)
Family type			
Nuclear	184	73.6	(62.3–80.1)
Joint	66	26.4	(21.1–32.1)
Marital status			
Married	145	58	(51.8–64.4)
Socioeconomic status			
Upper	2	0.8	(0.1–2.9)
Upper Middle	34	13.6	(9.6–18.5)
Lower Middle	100	40	(33.9–46.4)
Upper Lower	112	44.8	(38.5–51.2)
Lower	2	0.8	(0.1–2.9)
Ventilation			
Inadequate	81	32.4	(26.4–38.4)
Overcrowding			
Present	95	38	(32.1–44.3)
Close contact with case of TB			
Yes	77	30.8	(25.1–37.1)
Close contact with case of DR-TB			
Yes	20	8	(5.1–12.1)
Smoking			
Yes	60	24	(16.2–34.9)
Alcohol consumption			
Yes	55	22	(19.1–30.1)

based on the combined door and window area which when less than 2/5th of the total floor space area or in the absence of cross ventilation was considered to be inadequate.[12]

The patient interviews were conducted using a semi-structured questionnaire with both open ended and close ended questions and collected data on socio-demographic, environmental living conditions, alcohol and tobacco usage, treatment seeking behavior for TB in past and present. We also reviewed the patient case files and previous treatment cards to validate information. We assessed the socioeconomic status of the study subjects using the modified Kuppuswamy scale updated for income criterion.[13]

Ethics approval for the study was obtained from the National Centre for Disease Control, New Delhi and the same was approved by the hospital authority of RBIPMT.

The data was analyzed using Epi Info 7.2 (CDC, Atlanta). Univariate analysis was performed with the results for categorical data expressed in frequency and proportions while quantitative data was expressed in mean and standard deviation.

3. Results

A total of 4524 patients were admitted to RBIPMT with diagnosis of TB during the period of July–November 2013. Of these, we enrolled and interviewed a total of 250 patients with drug resistant TB; 198 (79.2%) were diagnosed as multidrug-resistant TB (MDR-TB) and 52 (20.8%) as extensively drug-resistant TB (XDR-TB). One sixty-five (66%) were males.

The median age of the patients with drug-resistant TB was 26 years, range 14–76 years (Table 1). A total of 47 (19 %) patients were illiterate and 133 (53.2%) were at-least high school graduates. As per the modified Kuppuswamy classification, 112 (44.8%) were classified as belonging to upper lower socioeconomic class followed by the lower middle (40%). There were 185 (74%) of the patients from nuclear family and 145 (58%) were married.

Seventy-seven (30.8%) patients gave history of contact with a confirmed case of TB prior to development of the disease in them while 20 (8%) reported contact with a confirmed DR-TB case. Overcrowding in the house and inadequate ventilation was reported by 95 (38%) and 81 (32.4%) of the patients respectively. A total of 24% (60) and 22% (55) of the patients reported history of smoking and alcohol intake respectively. Out of 250 patients screened for HIV, there were seven (3%) who had co-infection with human immunodeficiency virus (HIV).

The drug susceptibility testing with the line probe assay investigation results showed that among MDR-TB patients, the dominant drug resistance pattern was observed for isoniazid and rifampicin in 176 (70%) (Table 2). In case of XDR-TB, resistance to all the four first line drugs along with one Quinolone and one of the Injectable drugs was found in 41 (16%) cases.

Table 2 – Distribution of drug resistance pattern in study participants (*N* = 250).

Drug resistance pattern	Number	(%)	(95% CI)
MDR-TB			
HR	176	(70.4)	(64–76)
HRSE	12	(4.8)	(2–8)
HRS	9	(3.6)	(2–7)
HRE	1	(0.4)	(0.1–2)
XDR-TB			
HRSE + Oflox + KN/AM/CP	41	(16)	(12–22)
HR + Oflox + KN/AM/CP	6	(2.4)	(1–5)
HRS + Oflox + KN/AM/CP	5	(2)	(1–5)

The enrolled patients with DR-TB were treated for TB for a mean 2.3 times (range 1–6 times) before the establishment of the current diagnosis of DR-TB (Table 3). Eighty one (32.4%) patients took treatment from a private practitioner during the first episode of TB before coming to a government facility for availing DOTS therapy. All 81 were put on DOTS Category II for retreatment after coming to RNTCP (data not shown in table). At the beginning of TB treatment, almost all patients (99.2%) were diagnosed with pulmonary TB and only 2 had extra-pulmonary TB. During their entire treatment history, majority (58.4%) gave history of receiving treatment exclusively at government health facilities for DOTS while there were 98 (39.2%) patients who had received both DOTS and non-DOTS treatment.

Ninety-eight patients reported changing health facilities on their own without having been referred from the health facility; 63(64.3%) of whom did so due to the lack of improvement in their health status during the course of treatment. The inability to afford the escalating costs for continuing treatment was the second most common reason attributed to changing health facilities. Two hundred seventeen (87%) patients of drug-resistant TB reported previous enrolment for retreatment in DOTS category II. This includes all the patients (81/250) who received treatment from a private facility during their first episode of TB. These patients were classified as relapse in 102 (47%), failure in 37 (17%) and defaulter in 78 (35.9%) cases (Table 3).

Eighty-eight (35.2%) reported taking irregular anti-tubercular therapy. The patients attributed their behaviour to lack of money (36.4%), burden of work with risk of losing wages (35.2%) and side effects of the anti-tubercular medications ((23.9%). Most (86%) of the patients experienced one or the other adverse drug reactions due to anti-

Table 3 – Treatment history of study participants (*N* = 250).

Treatment factor	Frequency	Percentage	(95% CI)
Facility from where treatment received for first episode of TB			
Government	169	67.6	(61.4–73.4)
Private	81	32.4	(26.2–38.6)
Type of TB diagnosed for first episode of TB			
Pulmonary	246	98.4	(96.1–99.6)
Both pulmonary & EP	2	0.8	(0.1–2.8)
Extra pulmonary	2	0.8	(0.1–2.8)
Facility availed for treatment after TB diagnosis			
Only government (only DOTS)	146	58.4	(52.1–64.5)
Both Govt. & Private (both DOTS & non-DOTS)	98	39.2	(33.1–45.6)
Only private (only non-DOTS)	6	2.4	(1.1–5.1)
Reason for changing facility (*n* = 98)			
Not getting better	63	64.3	(54.1–74.1)
Couldn't afford treatment	33	33.7	(24.4–44.1)
Work timing	2	2.0	(0.25–7.2)
Regularity of treatment (*n* = 250)			
Regular treatment	162	65	(58.5–71.1)
Irregular treatment	88	35	(29.3–41.5)
Reason for irregular treatment (*n* = 88)			
Lack of money	32	36.4	(26.7–47.8)
Due to work burden	31	35.2	(24.6–45.4)
Side effect	21	23.9	(15.6–34.5)
Alcohol intake	3	3.45	(0.72–9.75)
Out of station	1	1.1	(0.03–6.24)
Reason for retreatment with Category II regime (*n* = 217)			
Relapse	102	47.0	(40.2–54.1)
Default	78	35.9	(29.6–42.7)
Failure	37	17.1	(12.3–22.7)
Reasons for default (*n* = 78)			
Lack of money	34	43.6	(32.4–55.3)
Due to work	20	25.6	(16.4–36.8)
Side effect	12	15.4	(8.2–25.3)
Not getting better	4	5.1	(1.4–12.6)
Alcohol intake	4	5.1	(1.4–12.6)
Felt better	2	2.6	(0.3–8.9)
Migration	2	2.6	(0.3–8.9)

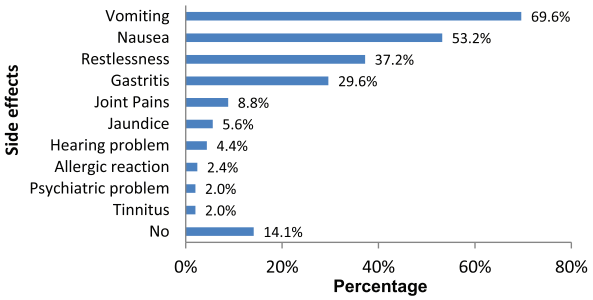

Fig. 1 – Adverse effects of anti-tubercular therapy reported by participants (N = 250).

tubercular treatment. Gastro intestinal symptoms (nausea, vomiting) were the most common side effect reported by the patients (Fig. 1).

The health services provided at the study site were found to be satisfactory in terms of convenient hospital service timings among 224(89%), cordial hospital staff among 245 (98%), regular and timely dispensing of medications along with an explanation of side effects of medicines at the time of dispensing among 226 (90%) of the patients.

4. Discussion

This study provides evidence on socio-demographic, environmental, baseline resistance pattern and health system related correlates for the patients with DR-TB admitted for MDR-TB treatment in a tertiary care TB hospital.

In our study, history of smoking and alcohol consumption was reported in nearly 25% of the patients almost all of whom were male. Smoking and alcohol consumption is also found to be associated with greater default in TB cases and increased risk of developing DR-TB.[14,15] Treatment outcomes in MDR-TB patients consuming alcohol is poor.[16]

Almost 87% of MDR-TB patients in our study were previously treated with category II under RNTCP. Other studies have also shown that previous treatment is a strong determinant of MDR-TB possibly due to amplification of resistance with retreatment in a few patients who fail first line therapy across the whole spectrum of adherence.[17–20] In our study among previously treated, 47% were relapse cases, indicating that a high proportion of relapse cases develop MDR-TB followed by default to treatment (35.9%) and treatment failure (17%). These findings are comparable to other studies and indicative of trends of MDR-TB among previously treated TB patients in Indian hospital settings.[19]

Poor adherence to anti-tubercular therapy increases chances of bacterial mutation in TB patients and leads to emergence of MDR-TB.[21,22] In our study, 35% patients reported taking irregular anti-tubercular therapy during their previous TB episodes. Reasons for irregular therapy were attributed by the patients to predominantly health system related factors. In our study, 64.3 % patients reported changing health facility either due to slow treatment response or non-affordable care (33.7%). Receiving multiple treatments either in public or private sector leads to increased variability in treatment and risk for emergence of MDR-TB.[23–25]

Our study showed that more than half of the patients (58.4%) who were treated exclusively in government facilities developed DR-TB. This has important programmatic implications. Inadequacies in the programme related to treatment access, non- flexible timing of public health institutes and absence of supportive counseling for side effects of anti-tubercular drugs are possibly affecting patient compliance to RNTCP DOTS. The RNTCP programme is using community DOT providers to improve access to treatment. Further incentives such as nutritional support and support for transportation cost might improve patient compliance and treatment outcome.

We also found that 32% of the patients made their first visit to a private practitioner for treatment of TB before coming to Government facility to receive DOTS treatment. Furthermore, a sizeable number continued to seek treatment from private sector even after TB diagnosis by RNTCP. The treatment imparted by private sector compared to RNCTP-DOTs often have high variability in quality with little improvement recorded in overall quality since the inception of the RNTCP.[26,27] Additionally, considerable out of pocket expenses are involved in seeking care from the private sector as opposed to free of cost DOTS from government health facility.

Most of the patients experienced one or the other adverse drug reactions. Gastro-intestinal symptoms were the most common side effect of treatment. Anti-tubercular medication side effects constitute a major factor responsible for poor adherence to tuberculosis treatment.[28] Healthcare providers should therefore promote awareness of side effects of drugs in TB patients and promote their ability to cope with them. In this regard, most of the participants found the functioning of the health services in the study site to be satisfactory.

There were certain limitations in the study. Data was collected based on the previous illness episode and hence subjected to recall bias. We did not critically evaluate the treatment pathway and delays from the point of initial TB diagnosis until the current initiation of DR-TB treatment at RBIPMT in those patients reporting treatment seeking from multiple healthcare providers. We collected information on DR-TB from referral hospital, leading to possibility of referral bias in patient selection. The quality of TB care delivered by private health care providers was also not assessed. Furthermore, due to the cross-sectional study design, treatment outcome on follow up could not be ascertained among the patients. Finally, since this was a quantitative study the complex social and behavioral factors involved with regard to the disease and its management could not be adequately assessed which require alternative qualitative approaches.

In conclusion, our study identifies important potential drivers for MDR-TB at patient level and health system level in a resource poor setting. Our findings have three important implications for India's RNTCP. One, category II previously treated should be considered a high risk for MDR TB. Therefore DST should be taken into account while deciding retreatment regimens for category II. Second, the programme needs to improve quality in service delivery to improve treatment adherence and strengthen follow-up and monitoring of patient progress during treatment to decrease default. Patient counseling should address personal level risk factors such as alcohol consumption, smoking and treatment side effects.

Third, private health sector continues to be an important health care delivery mechanism in India. Therefore, TB notification, anti-TB treatment regimens, monitoring and diagnostics have to be strengthened with strong linkages with private health care sector.

Conflicts of interest

The authors have none to declare.

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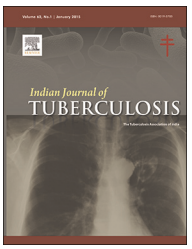

Original article

Study of IL-6 and vitamin D3 in patients of pulmonary tuberculosis

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ABSTRACT

Background: *Mycobacterium tuberculosis* can grow in hostile intracellular environment of macrophages by actively evading macrophage-associated antibacterial activities. The stress response factor contributes this process by releasing inflammatory cytokine Interleukin 6 (IL-6). IL-6 screening of patients with TB may be useful to monitor the progress of infection and to infer the risk of progression to active disease. Vitamin D has a critical role in the innate immune system, in the circulating metabolite and supports induction of pleiotropic antimicrobial responses, through the production of antimicrobial peptides, particularly cathelicidin and its active metabolite. 1,25-dihydoxyvitamin D, has long been known to enhance immune response to mycobacteria. In this study, we have studied the role of IL-6 and Vitamin D3 in *M. tuberculosis*.

Materials and methods: Three groups involved in this study are Control, Category I (newly diagnosed TB) and MDR TB patients. The serum levels of IL-6 and vitamin D3 were measured using chemiluminescence and fully-automated enzyme-linked immunosorbent assay respectively.

Results: The serum levels of IL-6 were significantly increased, whereas vitamin D3 decreased in TB multidrug-resistant group of patients compared to the newly diagnosed TB patients.

Conclusion: IL-6 appears to be the major cytokine elaborated by mycobacteria infection as well as play a role in the clinical manifestations and pathological events and hence may function as a potent biomarker of tuberculosis. Since, Vitamin D increases activity of cell-mediated immunity; it can be used as a supplementation during tuberculosis therapy.

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1. Introduction

The tubercle bacillus was discovered by Sir Robert Koch over a hundred years ago, yet tuberculosis remains one of the major health problems facing mankind. The emergence of drug-resistant organisms necessitates the development of new agents to enhance the response to antimicrobial therapy for active TB.

The significance of IL-6 production in tuberculosis is yet to be fully elucidated, although it is known for quite some time that IL-6 interferes with IFN-γ induced signal. Immunity to *Mycobacterium tuberculosis* is dependent upon the generation of a protective gamma interferon (IFN-γ)-producing T-cell response.[1,6,8–10,12,31] Recent studies have suggested that interleukin-6 (IL-6) is required for the induction of a protective T-cell response. As well as its role in the regulation of calcium metabolism, vitamin D is an immunoregulatory hormone.[9] In the preantibiotic era, TB of the skin was treated successfully with UV light. By the 1920s, pulmonary TB was being treated with regular sun exposure. During the last decade, laboratory research into the antimicrobial actions of vitamin D has provided new insights into these historical observations. Vitamin D has a critical role in the innate immune system through the production of antimicrobial peptides – particularly cathelicidin and its active metabolite, 1,25-dihydoxyvitamin D, has long been known to enhance the immune response to mycobacteria in vitro.[14] Vitamin D deficiency is common in patients with active TB, and several clinical trials have evaluated the role of adjunctive vitamin D supplementation in its treatment. Results of these studies are conflicting, reflecting variation between studies in baseline vitamin D status of participants, dosing regimens and outcome measures. Vitamin D deficiency is also recognised to be highly prevalent among people with latent *M. tuberculosis* infection in both high- and low-burden settings, and there is a wealth of observational epidemiological evidence linking vitamin D deficiency with increased risk of reactivation disease. Vitamin D could have an important role in the prevention and possible treatment of these conditions; however, much of the current evidence relates to basic science and epidemiological research. Randomised controlled trials of vitamin D supplementation for the prevention of active TB have yet to be performed, however. The conduct of such trials is a research priority, given the safety and low cost of vitamin D supplementation, and the potentially huge public health consequences of positive results.[2–5,17–30]

2. Material and methods

The subjects for this case–control study comprised normal human volunteers and pulmonary Tuberculosis, treated with dots blood samples were collected from all subjects and following parameters were estimated by fully auto ELISA (*Enzyme linked immunosorbant assay*) analyzer and Chemiluminescent Immulite 1000. Patients were of both gender, above 18 years but less than 60 years, from sir J.J. group of Hospitals, Mumbai outpatients department and admitted subjects were included in study. Patients who were un-cooperative or not willing to get enrolled in the study were excluded from study. Ethical Clearance approval taken from the institutional ethics committee of Grant Govt. Medical College and Sir J. J. Group of Hospitals, Mumbai and informed consent along with details of patients were taken prior to study. Blood samples were collected in plain vacutainer, centrifuge and serum sample were preserved in minus 80 degree centigrade and processed with Vitamin-D-ELISA Cat. No.: REA300/96, DLD Kit and Serum Cytokines IL-6- Chemiluminescent Immulite 1000, Siemens Medical Solutions Diagnostics, a solid- Phase, enzyme-labelled, chemiluminescent sequential immunometric assay. Statistical evaluation was done using ANOVA test using Minitab 17 software.

3. Results

In our study vitamin D levels were significantly low in MDR pulmonary tuberculosis as compared to control and category I in Table 5, when compare with age, sex wise distribution as in Table 1 with Graph 1 and correlation Table 2 and 3 with Graph 2 and 3 and socioeconomic status distributions in category I and multidrug resistant pulmonary tuberculosis Tables 8 and 9 and Graph 8 and 9.

Cytokines IL6 was significantly increased in MDR pulmonary as compare to control and category I Table 5, when compare with age, sex wise distribution as in Table 1 with Graph 1 and correlation Table 2 with Graph 2.

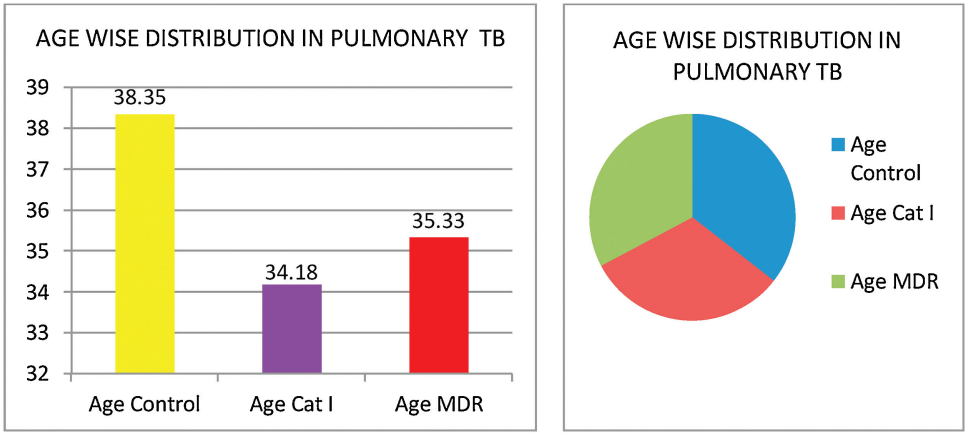
There is positive correlation between vitamin D levels in Table 4, Graph 4 and negative correlation in IL6 Table 6, Graph 6. Also negative correlation among vitamin D, Il6 Table 7 Graph 7.

4. Discussion

The TB epidemics create a health care crisis. The potential ability of vitamin D to fight TB, a leading cause of death could lead to decrease in mortality or slower the disease progression. Investing in TB control in poor settings can be a cost-effective approach. The connection among vitamin D, infections and immune function indicates a possible role for

Table 1 – Age and sex wise distribution in control and pulmonary tuberculosis.

Group	Age	Sex	
	Mean ± SD	Male	Female
Control (N = 100)	38.35 ± 13.14	50	50
Pulmonary tuberculosis			
Category I (N = 100)	34.18 ± 11.53	50	50
Multi drug resistant (N = 100)	35.33 ± 12.09	50	50



Graph 1 – Bar graph and pie graph of age and sex wise distribution in pulmonary TB.

Table 2 – Correlations between age in control and pulmonary tuberculosis.

Group	*r*-Values	*p*-Value
Control/category I Pulmonary tuberculosis	0.090	0.638
Control/multi drug resistance	0.082	0.666
Category I/multi drug resistance	0.085	0.654

Table 3 – Vitamin D levels in control and pulmonary tuberculosis.

Group	Vitamin D levels in ng/mL Mean ± SD
Control (*N* = 100)	51.21 ± 4.31
Pulmonary tuberculosis	
Category I (*N* = 100)	27.54 ± 3.59
Multi drug resistant (*N* = 100)	5.48 ± 3.36

Table 4 – Correlations between vitamin D control and pulmonary tuberculosis.

Group	*r*-Values	*p*-Value
Control/category I Pulmonary tuberculosis	0.970	0.000
Control/multi drug resistance	0.937	0.000
Category I/multi drug resistance	0.912	0.000

vitamin D supplementation.[4,7,8,11,18,20] A study tested the association between vitamin D deficiency rickets and protein-energy malnutrition (PEM) in Ethiopian children and suggested that programmes targeting vitamin D deficiency

Table 6 – Correlations between cytokine IL6 in control and pulmonary tuberculosis.

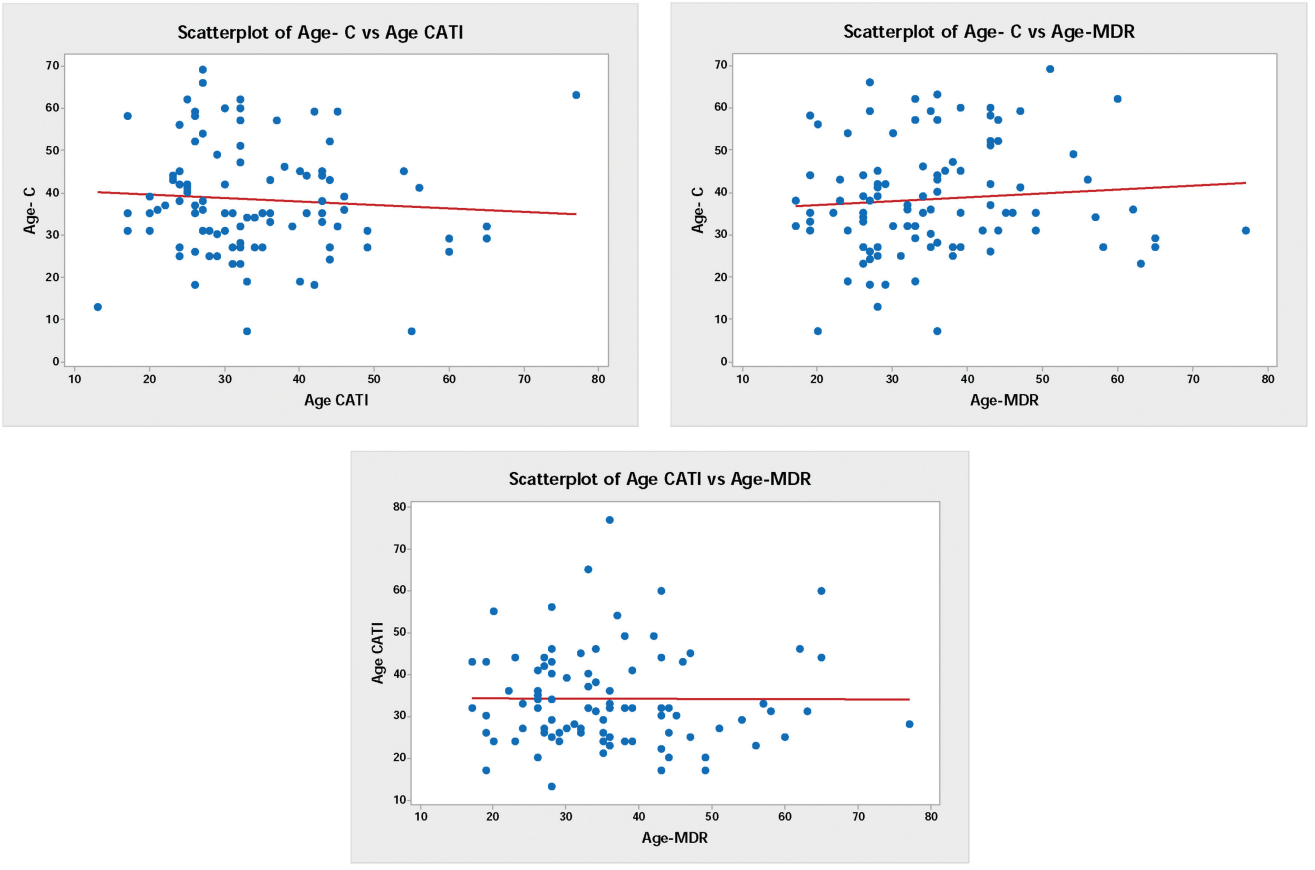
Group	*r*-Values	*p*-Value
Control/category I Pulmonary tuberculosis	−0.028	0.883
Control/multi drug resistance	−0.124	0.512
Category I/multi drug resistance	−0.110	0.561

Table 7 – Correlations between vitamin D and IL6 in control and pulmonary tuberculosis.

Group	*r*-Values Vit D/IL6	*p*-Value
Control/control Pulmonary tuberculosis	0.040	0.833
Control/category I	−0.015	0.937
Control/multi drug resistance	−0.563	0.001
Category I/category I	0.044	0.817
Category I/multi drug resistance	−0.526	0.003
Multi drug resistance/multi drug resistance	−0.757	0.000

Table 5 – Cytokine IL6 levels in pulmonary tuberculosis.

Group		IL6 levels in pg/mL Mean ± SD
Control (*N* = 100) Pulmonary tuberculosis		3.50 ± 0.87
Category I	Total of low and high values (*N* = 100)	7.38 ± 17.74
	Low values (*N* = 86)	3.44 ± 1.69
	High values (*N* = 17)	44.82 ± 41.61
Multi drug resistant	Total of low and high values (*N* = 100)	91.69 ± 138.68
	Low values (*N* = 68)	23.39 ± 0.90
	High values (*N* = 32)	234.73 ± 157.82



Graph 2 – Correlations between age in control, category I and MDR pulmonary TB.

rickets should give emphasis to children with PEM.[13,14] The relationship between rickets and maternal vitamin D deficiency has important implications for a comprehensive prevention strategy of vitamin D deficiency in women and children in the poorest settings of developing countries.[21,22] Moreover, the potential health consequences of vitamin D are very crucial in Africa and Asia where the infectious disease burden is high and malnutrition and famine are spreading in many populations, lowering their immune system.[19] Therefore programmes should be planned in order to provide vitamin D mainly to pregnant women and children. In the western countries, more attention should be paid to the nutritional and vitamin D needs of older people and of all high risk groups (immigrants, HIV subjects, TB patients) who are prone to hypovitaminosis D.[12,14,17,21,24–30] Public health education should stress the need for adequate dietary intake of vitamin D in spite of good exposure to sunlight in vulnerable groups of people all over the world.

Table 8 – Socioeconomic status distributions in category I pulmonary tuberculosis.

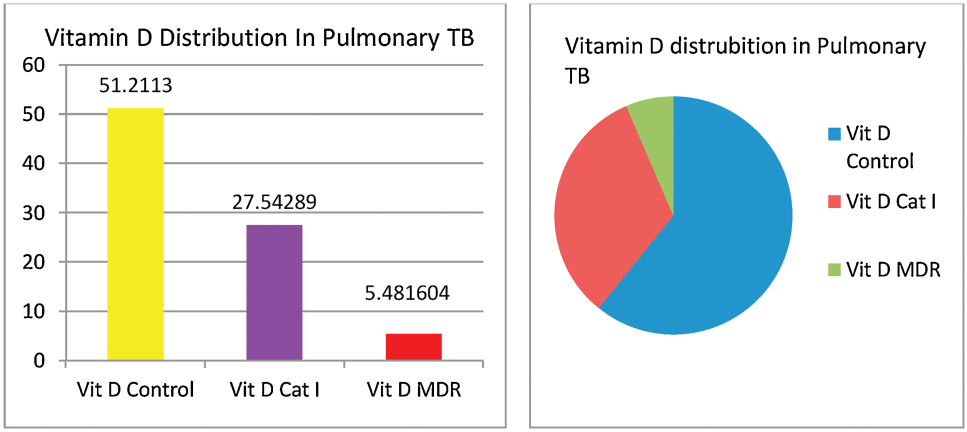
Socioeconomic class	Number of cases
I	0
II	4
III	45
IV	29
V	22

Table 9 – Socioeconomic status distributions in multidrug resistant pulmonary tuberculosis.

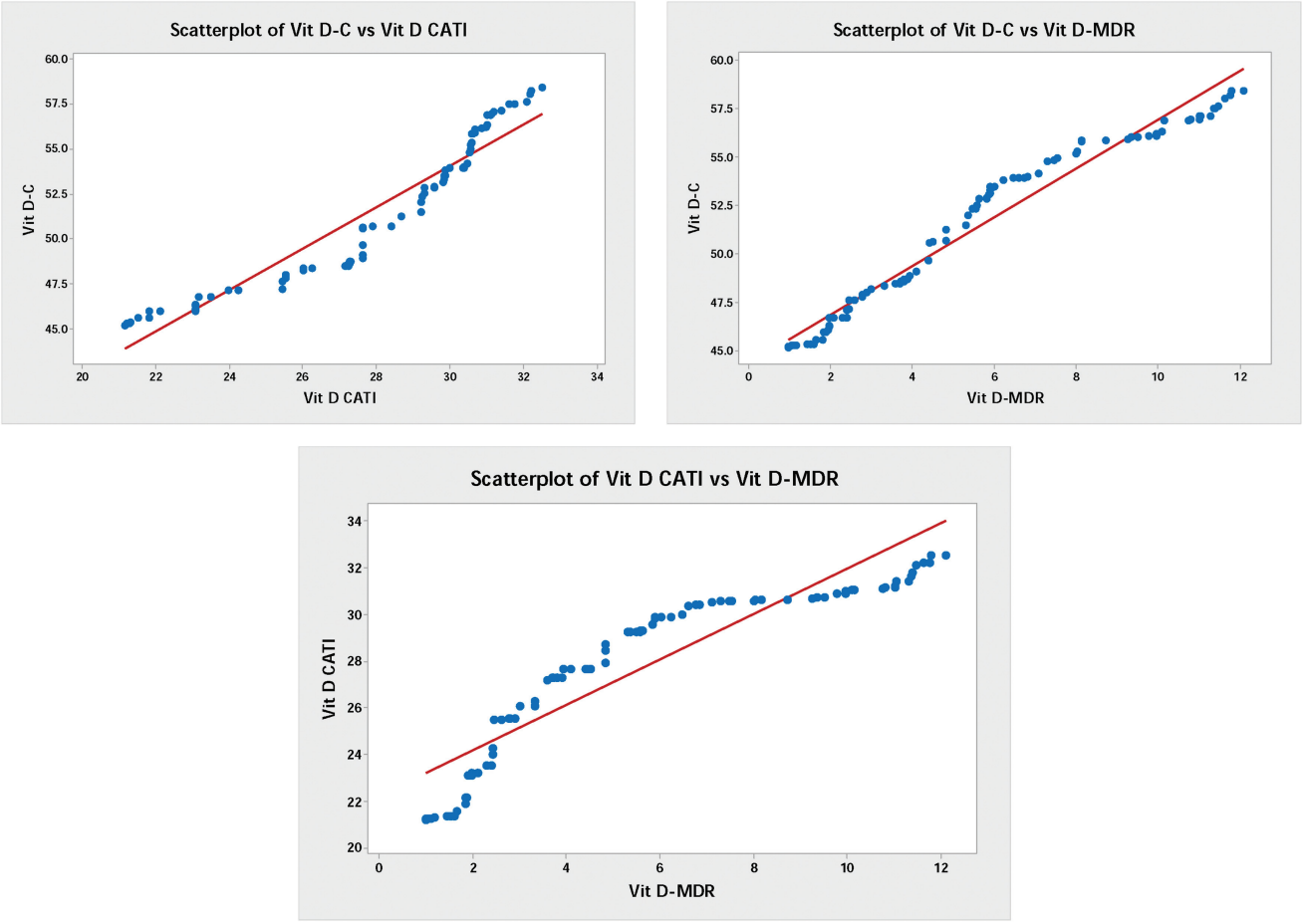
Socioeconomic class	Number of cases
I	0
II	5
III	41
IV	37
V	17

Present study noticed that cytokine IL6 plays a major role in the pathogenesis of tuberculosis. The estimation of cytokines IL6 levels and fat soluble vitamin D gave an idea about the possibility of tissue damage while the levels of vitamin D indicated deficiency in tuberculosis patients.

All the control subjects as well as study groups belong to poor nutritional status and low socio economic status from Mumbai. These subjects also found to have common habit of sharing of clothes, food, drinking water container, tea etc. without broughening of spread of infection. In history taking it



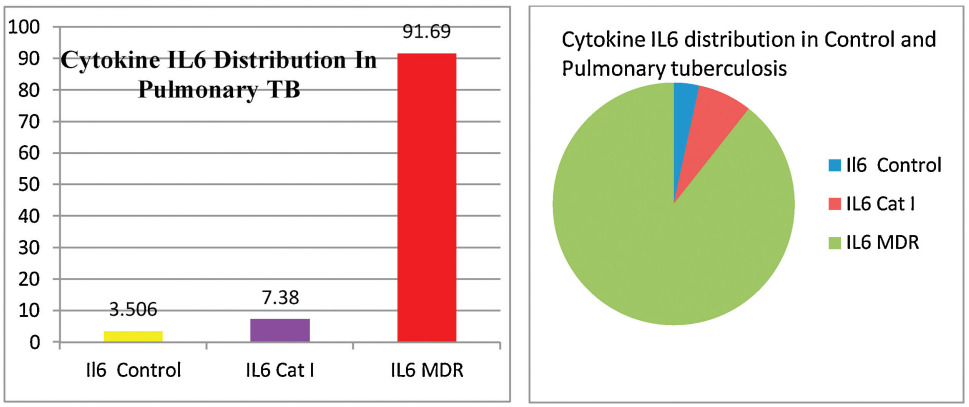
Graph 3 – Bar and pie graph vitamin D levels in control and pulmonary tuberculosis.



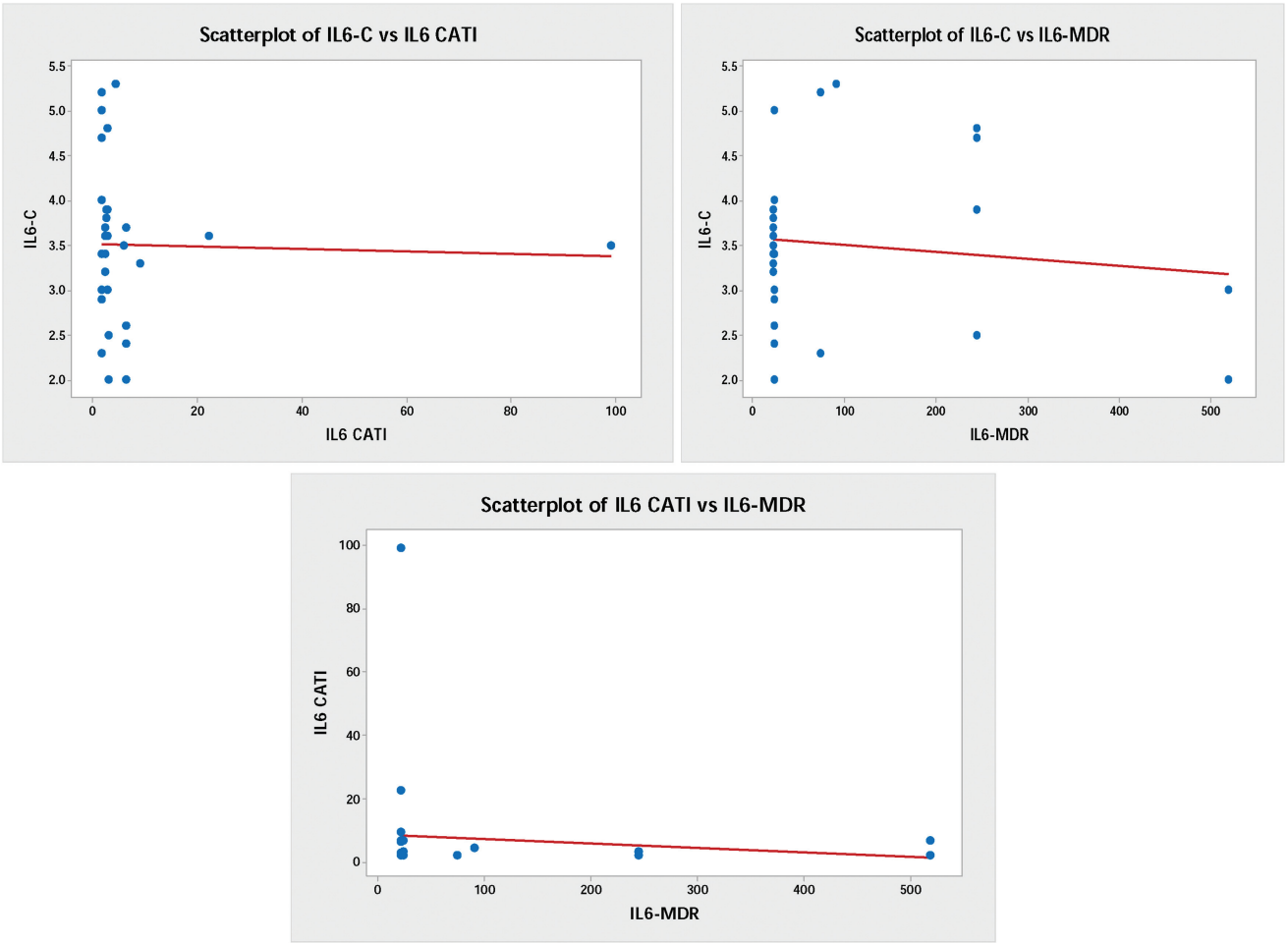
Graph 4 – Correlations graph between vitamin D-control, category I and MDR TB.

is also found that they are closer to one another (sharing of small space) which lead to the close contact breathing (aerosol contamination), coughing and sneezing lead to spread of infection.

Cytokine level could be used as an index of IL6 in various categories of pulmonary tuberculosis. It could be applied to judge the clinical severity of the disease. The functions of vitamin D are complementary to each other and overall

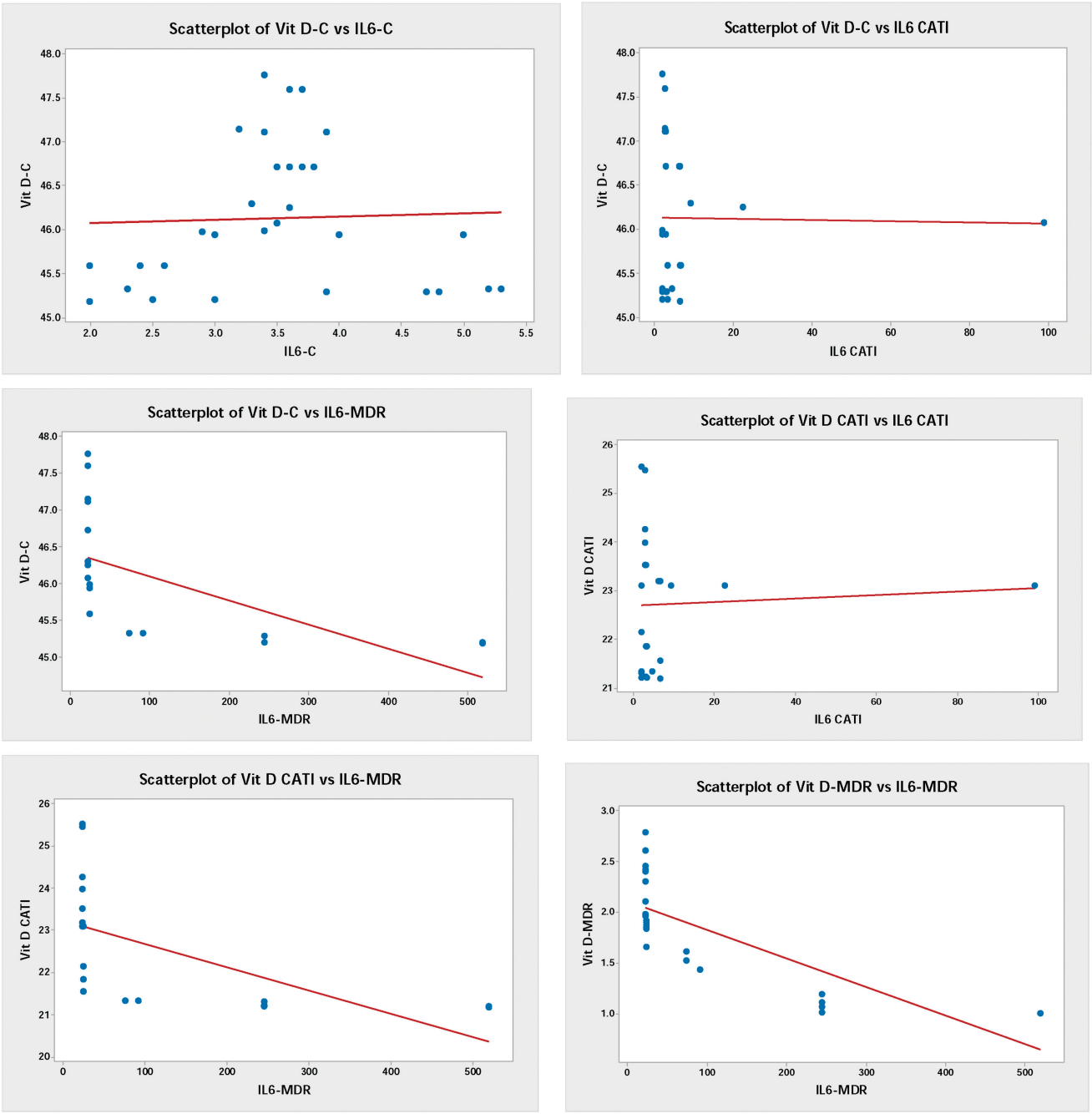


Graph 5 – Bar graph and pie graph cytokine IL6 levels in control and pulmonary TB.



Graph 6 – Correlations between IL6-control, CAT I, MDR in pulmonary TB.

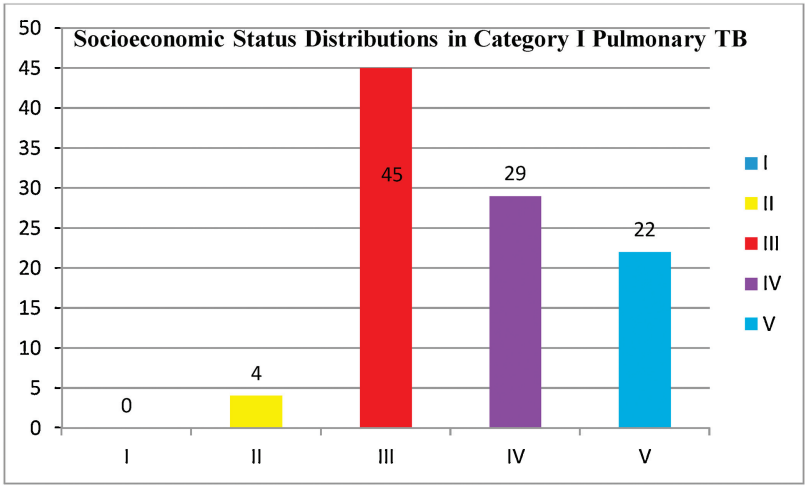
deficiency of these vitamins or hormone could inhibit their sparing actions on each other resulting in uncontrolled increase in IL6 and showing ageing.[15,16,31] The present study show low levels of Vitamin D as disease progress and increase in established a good negative correlation between IL6 levels and vitamin D, elucidating the importance of dietary vitamins in preventing the oxidative damage in tuberculosis and their deficiency can be used as sensitive indicator of oxidative stress in tuberculosis patients. This fact also gave us an idea about supplementation of vitamins along with anti-tuberculosis



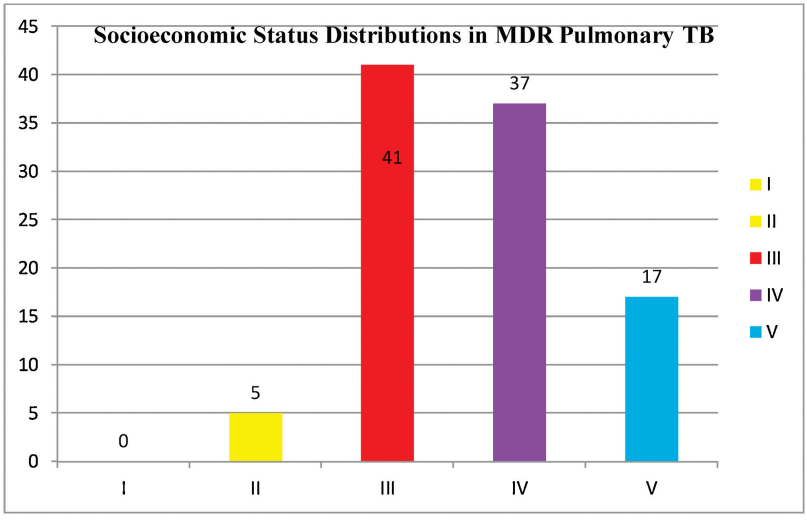
Graph 7 – Correlation of vitamin D and cytokine IL6 of control, category I and MDR in pulmonary tuberculosis.

drugs. The tissue enriched with such endogenous vitamin D could be more effective in dealing with stress due to oxygen in tuberculosis as well as vitamin supplement help to prevent general metabolic related sluggishness associated with tuberculosis related complication. The therapeutic efficacy of this endogenous vitamin along with anti-tuberculosis drug therapy may help to prevent complication in tuberculosis. In depth study at gene polymorphisms study at molecular level is required to correlate the pathogenesis and progression of multidrug resistant in tuberculosis disease with Cytokines and Vitamin D along with drug treatment.[6,8,10,12,15,16]

Preventive measure need to be followed in the people having poor nutrition and lower socioeconomic status for awareness, mass education needed to train them to take complete course of treatment also supplementation of vitamins (according to blood levels) required.



Graph 8 – Socioeconomic status distributions in category I pulmonary tuberculosis.



Graph 9 – Socioeconomic status distributions in multidrug resistant pulmonary tuberculosis.

5. Conclusion

Increase oxidative stress and nitrosative stress, leading to increase in cytokine levels in tuberculosis. The increased Il6 levels hampers many important functions and show early ageing process. Future Clinical Trials: can Vitamin D therapy alter the natural history of disease in the age of XDR-TB.

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Conflicts of interest

The authors have none to declare.

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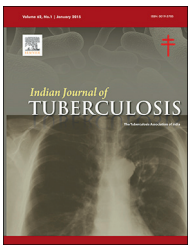

Original Article

Bilateral Tubercular Dactylitis: Unusual presentation of an usual disease

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ABSTRACT

Introduction: Bilateral Tubercular Dactylitis (TD) is an unusual presentation of tuberculosis and only handful numbers of cases are reported in the literature. Hence, very little is known about its clinical presentation, statistic, radiological features and its outcome.
Methods: We have included seven male and two female patients of mean age 7.2 years, of the proven cases of bilateral TD by histopathological or microbiological or PCR analysis from core biopsy. Radiological features were recorded from plain radiograph. All patients were given Antitubercular drugs according to WHO 2010 recommendation (four drugs for 3–5 months, three drugs for next 3–5 months and finally two drugs for 6–8 months). Debridement was done whenever required.
Results: Of total 26 lesions, the most common presentation was swelling with or without mild pain. Discharging sinus was present in four lesions. There were six phalanges, 18 metacarpal and two metatarsals. Radiographically, the most common type of lesion was soft tissue swelling followed by lytic lesion. Histopathologically tuberculosis was proven in 10 (55.6%) lesions, bacteria isolated in 5 (27.8%) lesions and PCR was done in 8 lesions and was positive in all. All lesions healed after giving ATT except one which developed psudo-arthrosis and one patient developed coronal plane deformity that was corrected by JESS.
Conclusion: A clinician should always suspect tuberculosis while dealing with a pathology of hand and feet even if it is bilateral. Suspected case can be diagnosed by histopathological, microbiological or PCR analysis and it can be treated by ATT with a good functional outcome.

1. Introduction

Tubercular Dactylitis (TD) is a rare form of osteoarticular tuberculosis that involves short tubular bones of hand and feet (phalanges, metacarpals and metatarsal).[1] It affects the age group of less than 6 years in majority of the cases (approx 85%) due to lavish blood supply through a large nutrient artery entering almost in the middle of the bone. Most of the reported cases are unilateral with or without pulmonary involvement.

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Multifocal TD usually occur in immunocompromised patients but it may occur in the patients with normal immunity.[2] Only few scattered case reports of bilateral TD are available in the literature and hence, very little is known in terms of its statistic, clinical features and final outcome.

In this prospective observational study we have tried to highlight the distribution statistics, clinical features, radiological features and final outcome of the TD involving bilateral extremities.

2. Material and methods

This was a prospective observational study done in between January 2011 and December 2014 in two tertiary referral centre. The inclusion criteria included patients with a proven tubercular osteomyelitis by histopathological or microbiological analysis. The patients who attended our outpatient or inpatient department with bilateral TD were included in the study. There were seven males and two females with the mean age of 7.2 years (2–14). A data sheet was prepared including all demographic details. A detailed history and examination were recorded. Plain radiograph in two plains were taken in all cases at the start of treatment and also at subsequent follow-up. Plain radiological features of the lesion were described as lytic lesion (L), ballooning (B), periosteal reaction (P), soft tissue swelling (S), cortical erosion (E), sequestrum (Sq) and sclerosis (Sc). MRI was done in the case where plain radiograph showed no abnormality but clinically the patient had symptoms highly suspicious of tuberculosis. Chest radiograph were also taken in all patients to rule out active or healed pulmonary tuberculosis. A complete blood panel including cell counts, Erythrocyte Sedimentation Rate (ESR), CD-4 count C-reactive Protein (CRP) and baseline liver function enzymes were performed in all cases. Core biopsy/open biopsy was done in all patients from a representative lesion from both extremities and samples were sent for gram staining, culture and sensitivity, acid fast staining and histopathological analysis. In equivocal cases PCR was done. Confirmation of the diagnosis was done on the basis of characteristic histopathology, a positive culture for Mycobacterium tuberculosis and/or a positive smear for acid fast bacilli or positive PCR analysis.

All patients were treated as per the recommendations of WHO 2010 which categorised osteoarticular TB as a severe form of TB in category I. The anti tubercular treatment (ATT) regimen was followed using four drugs (rifampicin, Isoniazid, Pyrazinamide and ethambutol) for intensive phase (3–5 months), three drugs (Isoniazid, rifampicin and Pyrazinamide) for 3–5 months in continuation phase and two drugs (Isoniazid and Rifampicin) for (6–8) months in maintenance phase. The duration of therapy which is a grey zone in the treatment protocol varied from a duration of 12 to 18 months depending upon the signs of radiological healing or settlement of metabolic inflammatory parameters (ESR, CRP). In case of patients presenting with painful lesions plaster slabs or splints were given for short period of time in order to relieve acute symptom for variable duration depending upon the resolution of symptoms.

Operative treatment by debridement (with or without curettage) was performed in the cases of discharging sinus on presentation and who was not responding to ATT after six to eight weeks of treatment. All the patients were followed up regularly and radiograph and blood investigations were also done to check for the response of treatment.

3. Results

Out of total nine patients, there were two female and seven male with mean age of 7.2 years (2–14). There were five patients whose age was more than or equals to six years. Most common presentation was swelling (Fig. 1(a)) with or without mild pain. Axillary lymphadenopathy was present in one patient (case no. 2, Fig. 1(b)) and cervical lymphadenopathy in one patient. Discharging sinus was present in three patients (four lesions) (case no. 4, 6, 8). Based on plain radiograph and MRI, there were total 26 bone involved in nine patients, 12 in right and 14 in left side. Out of 26 bones, there were six phalanges (23%), 18 metacarpals (69.2%) and two metatarsals (7.8%) (Table 1). Multifocal systemic involvement (pulmonary, cervical and dorsal spine) was there in one (1.1%) patient (case no. 4, Fig. 1(c–e)). Out of nine patients only two (22.2%) had constitutional symptoms like mild grade of fever, decreased appetite and significant weight loss. Three (33.3%) patients gave history of tuberculosis contact.

On plain radiograph, types of the lesion were lytic in 14 (53.8%), ballooning in 8 (30.7%), periosteal reaction in 5 (19.2%), soft tissue swelling in 16 (61.5%), cortical erosion in 3 (11.5%), sequestrum in 1 (3.8%) and sclerosis in 8 (30.7%) (Table 2). The most common radiological feature was soft tissue swelling (16; 61.5%) followed by lytic lesion (14; 53.8%). The most common radiological feature in phalanges was also soft tissue swelling followed by lytic lesion, whereas in metacarpal or metatarsal most common feature was lytic lesion followed by soft tissue swelling. One patient (one lesion) required MRI because plain radiograph showed no bony abnormality (Fig. 2(a–c)). A core biopsy was performed in all patients from a representative lesion of both extremities (18 lesions). Histopathological features suggestive of tubercular aetiology were seen in 10 (55.6%) out of 18 biopsy taken. Isolation of bacteria was possible from 5 (27.8%) out of 18 lesions and all were sensitive to first line antitubercular drugs. PCR was required for the diagnosis in eight lesion and was positive in all (Table 1). Blood investigation reports showed lymphocytosis in five (55.6%) patients, raised ESR in seven (77.8%) patients and normal CD-4 counts in all patients.

Conservative management in the form of ATT with or without splint was successful in six patients (Fig. 3(a–f)), debridement were required in three patients (four lesions) as there were no improvement after four weeks of ATT. Radiographically, all except one lesion healed with sclerosis irrespective of type of lesion observed at the start of treatment. In one lesion pathological fracture after debridement occurred and final healing took place with a pseudoarthrosis (Fig. 4). Finally healing without complications were

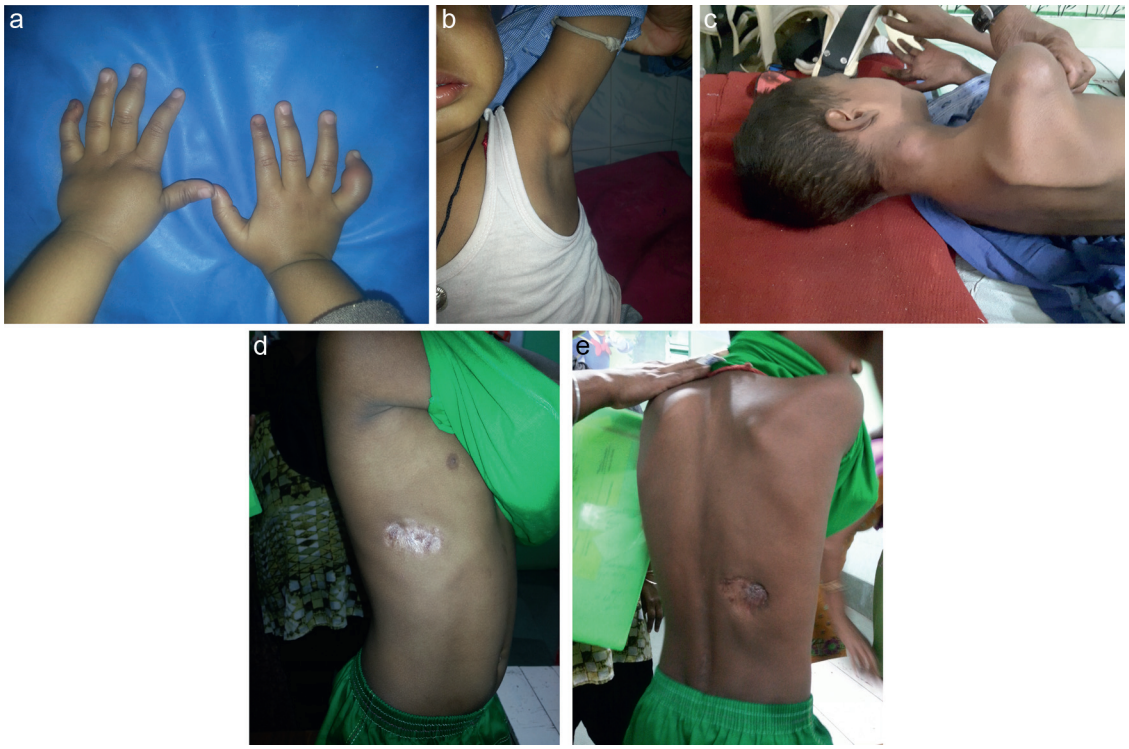

Fig. 1 – Showing different clinical features: (a) Swelling of fingers; (b) Axillary lymphadenopathy; (c) Cervical region involvement; (d) Pulmonary involvement; (e) Dorso-lumber involvement. Figure (c–e) was of the same patient (case no. 4) having multifocal systemic involvement.

seen in seven patients, one patient developed pseudarthrosis of metacarpal and one had a deformity in coronal plain that was further corrected using Joshi External Stabilization System (JESS) fixator. Although patient had pseudarthrosis, the hand function score measured using the modified score of Green and O'Brien which was 80 points. The mean hand function score in left side was 90.5 (80–95) and on right hand was 90 (80–95). For the assessment of foot function no score was used, but subjectively she had excellent result (case 6).

4. Discussion

TD constitutes 0.65–6.9% of all forms of tuberculosis cases in children.[3] The diagnostic delay in children is attributed to a lot of factors including the lack of suspicion among clinicians, nonspecific wide variations in clinical manifestations. Simultaneous involvement of both the limbs together tilting the diagnostic thinking away from infective aetiology and also presentation at an unusual age as tubercular osteomyelitis is uncommon beyond 6 years of age, once the epiphyseal centres are well established and also since most of the lesions associated with tubercular dactylitis is paucibacillary this adds to the diagnostic difficulty.[4] It is more common in younger population with 85% cases in children younger than 6 years.[3] In contrast to above fact, there were five (55.6%) patients with the age more than or equal to 6 years. TD is reported to be three times more common in male than female.[5] In this case series male to female ratio was 3.5:1. It has been found that low socioeconomic status is a most common risk factor for TD, but immunodeficiency is the most important risk factor for dissemination of tuberculosis to hands and feet.[6–8] In our case series there were seven patients (77.8%) who belonged to a low socioeconomic group. TD of hands is reported more common than the feet, similar to this series where only one patient had TD of foot (two lesions, both of metatarsal). There were no lesions involving the distal phalanx, well supported by literature.[6]

The main presenting complaint of TD is swelling with or without pain not responding to analgesic[3,9,10] followed by discharging sinus in 20% of the cases.[11] Swelling is usually very less remarkable in foot because bones of feet are covered by tendon and tight fascial sheaths which resist distension.[12] The TD always pose a diagnostic dilemma primarily due to insidious onset, slow progression, minimal symptoms and nonspecific clinical presentation, non-specific nature of radiographic findings, paucity of rapid diagnostic test to confirm it and sometime inconclusive histopathological and microbiological observation, and finally little awareness among treating physician who do not have much experience of TD.[9–11,13–18] Diagnosis of TD is based primarily on the clinical and radiographic pictures, and should be confirmed by microbiological or histopathological examination.[17,19]

Blood examination may show elevated ESR in between 60 to 88% of cases.[9,20] Pathognomonic radiographic feature

Table 1 – Descriptive data of all patients.

Case no.	Age (years)	Gender	Side	Location of lesion	Clinical features	Radiological features	Histopathology	Isolation of bacteria	PCR	Treatment	Follow-up (in months)	Outcome
1	2	Male	Rt	Middle Phx little finger	Swelling	L, S	+	–	ND	Conser.	18	Healed
			Lt	Middle Phx. middle finger	Swelling	S, Sc	+	+	ND	Conser.		Healed
				Second MC	Swelling	S, Sc	ND	ND	ND	Conser.		Healed
				Fifth MC	Swelling	L, P, B	ND	ND	ND	Conser.		Healed
2	4	Male	Rt	First MC	Swelling	S, P, Sc	–	–	+	Conser.	27	Healed
				Fourth MC	Swelling	S, P, Sc	ND	ND	ND	Conser.		Healed
			Lt	Proximal Phx Middle Finger	Swelling with axillary lymphadenopathy	L, B, S, E	–	–	+	Conser.		Healed
				Fifth MC		L, B	ND	ND	ND	Conser.		Healed
3	6	Male	Rt	Fourth MC	Swelling	Sc	+	+	ND	Debri.	22	Healed
				Middle Phx Middle finger	Swelling	Sc, S	ND	ND	ND	Conser.		Healed
			Lt	Third MC	Swelling	L, B	+	+	ND	Conser.		Healed
4[a]	10	Male	Rt	Fourth MC	Swelling	L, B, E	+	–	ND	Conser.	29	Healed
				Fifth MC	Swelling	L, B, E, S	ND	ND	ND	Conser.		Healed
			Lt	Middle Phx index finger	Swelling with discharging sinus	P, S	–	–	+	Debri.		Healed
5	5	Male	Rt	Second MC	Swelling	L, S	–	–	+	Conser.	30	Healed
			Lt	Fourth MC	Swelling	S, Sc	ND	ND	ND	Conser.		Healed
				Fifth MC	Swelling	L, P, B	–	–	+	Conser.		Healed
6	14	Female	Rt	Fifth MT	Discharging sinus	L, E	+	–	ND	Debri.	28	Healed
			Lt	First MT	Discharging sinus	L, Sq	+	+	ND	Debri.		Healed
7	12	Male	Rt	Second MC	Swelling and pain	N	–	–	+	Conser.	15	Healed
			Lt	Middle Phx index finger	Swelling and pain	N	–	–	+	Conser.		Healed with coronal plain deformity
8	8	Female	Rt	Second MC	Swelling with discharging sinus	L, B, S	+	+	ND	Debri.	18	Healed with psudoarthrosis
			Lt	Fifth MC	Swelling	L, S	+	–	ND	Conser.		Healed
9	4	Male	Rt	Proximal Phx Middle Finger	Swelling	L, S	+	–	ND	Conser.	17	Healed
			Lt	Third MC	Swelling	L, S	–	–	+	Debri.		Healed
				Fifth MC	Swelling	Sc	ND	ND	ND	Conser.		Healed

Keywords: Rt = right; Lt = left; Phx = phalanges; MC = metacarpal; MT = metatarsal; L = lytic lesion; B = ballooning; P = periosteal reaction; S = soft tissue swelling; E = cortical erosion; Sq = sequestrum; Sc = sclerosis; N = no abnormality; ND = not done; Conser. = conservative; Debri. = debridement.

[a] Case with multifocal systemic involvement.

Table 2 – Radiological features.

Type of radiological lesion	Lesions affected	Percentages
Lytic	14/26	53.8%
Ballooning	8/26	30.7%
Periosteal reaction	5/26	19.2%
Soft tissue swelling	16/26	61.5%
Cortical erosion	3/26	11.5%
Sequestrum	1/26	3.8%
Sclerosis	8/26	30.7%
No abnormality	2/26	7.6%

of TD is ballooning of the bone called as ''Spina Ventosa'', but it is seen only in nearly 50% of the cases.[6] In our series ballooning was observed only in eight lesions (30.7%). Other radiographic findings are soft tissue swelling, lytic lesions, cystic expansion of the bone, cortical erosion, cortical destruction, minimal periosteal reaction, sclerosis in long standing cases, presence of sequestrum, reduction of adjacent joint space, subchondral erosion and pathological fracture, and combination of above all.[17] In our study, soft tissue swelling was the most common radiological finding followed by lytic lesion.

Isolation of Mycobacterium tuberculosis is possible in 25–75% of the cases of osteoarticular tuberculosis depending upon the culture material available. We observed culture positive cases only in 33.3% of the cases. The histopathological diagnosis of osteoarticular tuberculosis is reported in between 72–97%, and it is characterised by presence of caseous necrosis, epithelioid cell granuloma and Langerhans giant cells.[21] The osteoarticular tuberculosis is a paucibacillary and hence PCR is considered to be an excellent diagnostic tool. It has been reported positive in 98% of the cases.[21] We observed it positive in all samples sent for PCR.

The differentials that should be kept in mind while dealing with a suspected case of TD are dactylitis in sickle cell disease, histiocytosis X, hereditary acro-osteolytic conditions, syphilis, pyogenic osteomyelitis, fungal infection, and tumours.[22] Out

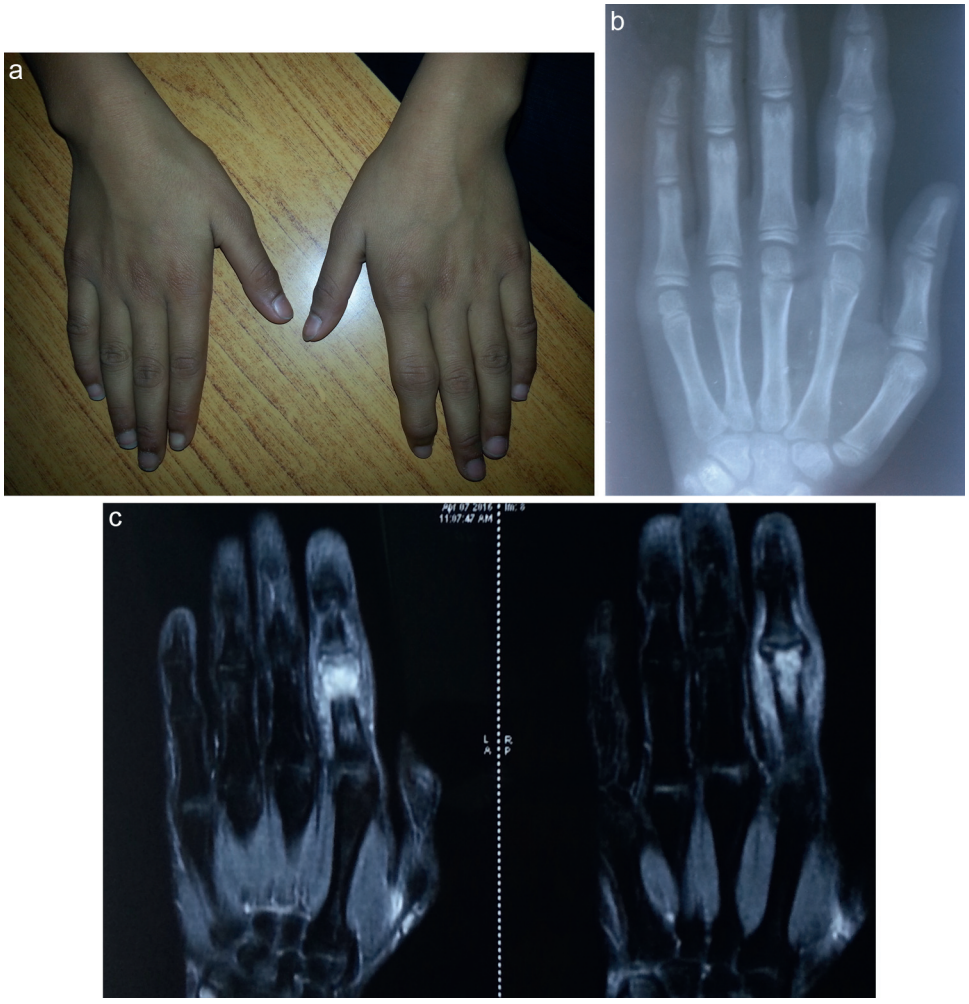

Fig. 2 – (a) Showing swelling around distal interphalangeal joint of index finger of the left hand, (b) showing plain radiograph of the same patient, was of not much informative except minimal soft tissue swelling, (c) showing its MRI having signal changes in the distal part of middle phalanx of index finger and its histopathology after core biopsy was suggestive of tubercular origin.

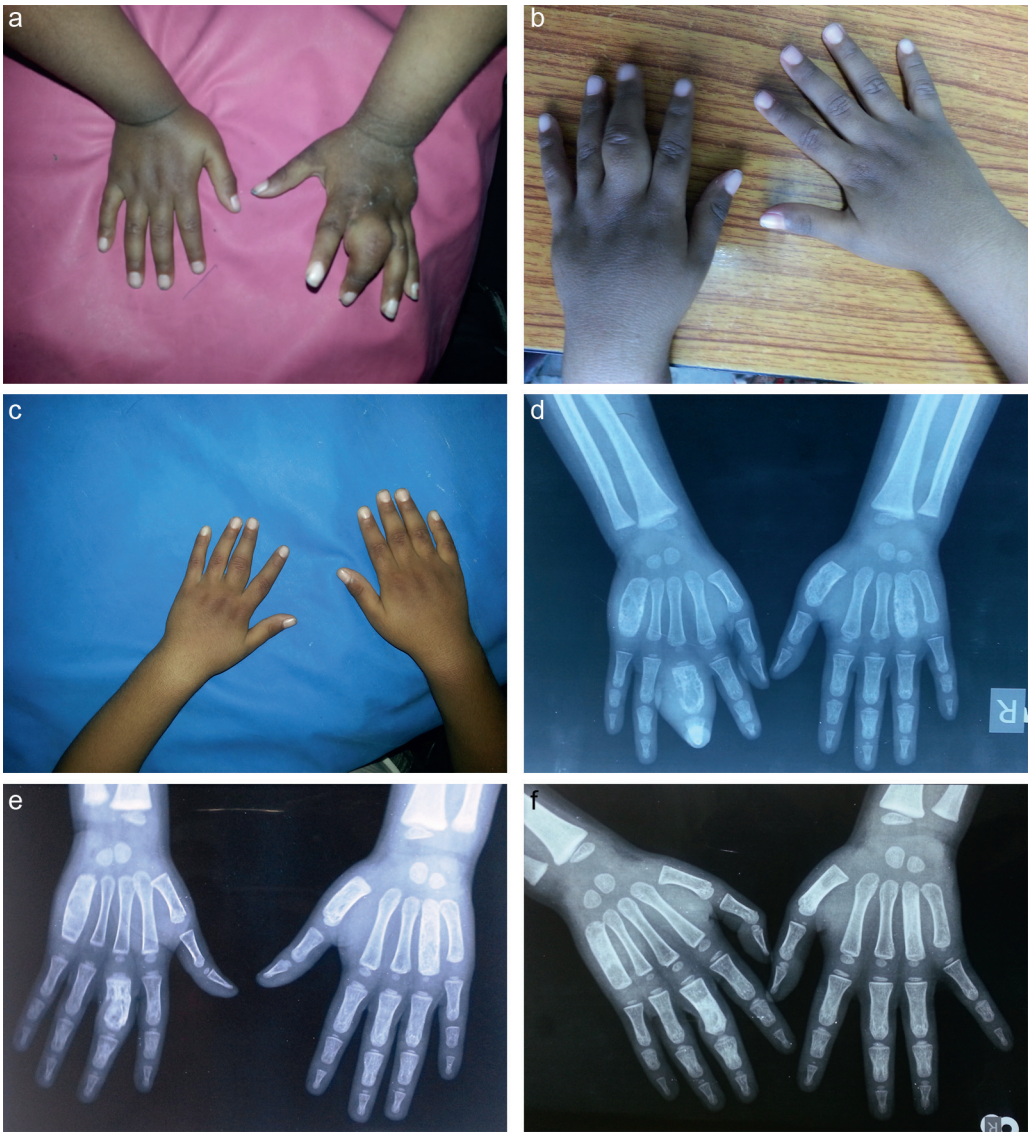

Fig. 3 – Healing of the lesions. (a–c) Clinically, (a) at the start of treatment, (b) 6 months after start of treatment, (c) at the completion of treatment. (d–f) Radiologically, (d) at the start of treatment, (e) 6 months after start of treatment, (f) at the completion of treatment.

of these bilateral involvement is seen in sickle cell disease, histiocytosis X and hereditary acro-osteolytic conditions. In syphilis, the bone is thickened by periosteal reaction. Pyogenic osteomyelitis tends to be associated with high grade fever, acute swelling, painful and hot. In sickle cell disease, the feature is similar to that of TD and characterised by bilateral involvement and dissolution of the sickle cell lesion is typically followed by irregularly sclerotic new bone formation.[23] To rule out malignant conditions histopathological examination is essential.

In most of the cases, conservative treatment is sufficient for the treatment of tubercular osteomyelitis. There are numbers of ATT regimens are described in the literature regarding its combination and duration of the treatment required in osteoarticular tuberculosis. Operative treatment in the form of debridement or drainage is required if not responding well to a conservative treatment. However, there are recommendation that debridement of the lesion can be helpful in prompt tissue diagnosis and rapid recovery with ATT.[7,24] In this study debridement was done in four bones that were not responding to conservative treatment. After debridement all lesions healed. We had achieved successful treatment in 77.8% (seven patients) of the cases with good functional outcome after conservative method of treatment. This observation is well supported by the study of Kotwal et al.[25] who reported good functional outcome in 75% of cases after conservative management. There are few limitations of this study like small number of sample size.

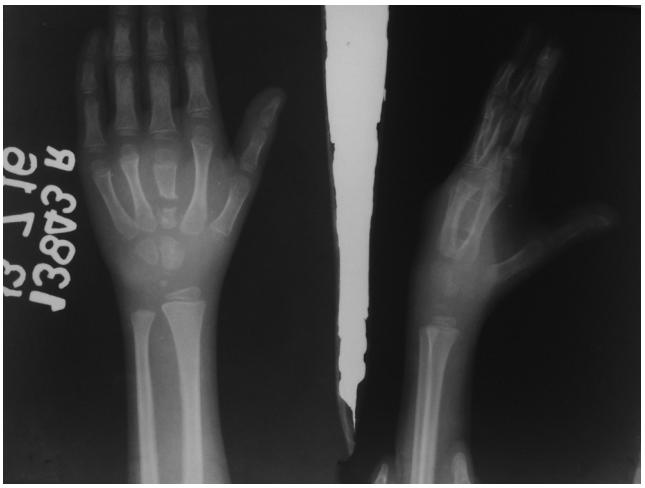

Fig. 4 – Showing psudoarthrosis of the third metacarpal after debridement and pathological fracture, but his hand function was reasonably good.

5. Conclusion

In a tubercular endemic country like India, a clinician should always suspect TD while dealing with the pathology of hands and feet even if there is bilateral involvement as tuberculosis can present in the most unusual form in the most unusual site. Suspected case should undergo radiological, microbiological and histopathological examination before start of treatment. TD can be successfully treated with good functional outcomes after a full course of antitubercular drugs, and if required operative treatment should be considered in nonresponsive lesions.

Conflicts of interest

The authors have none to declare.

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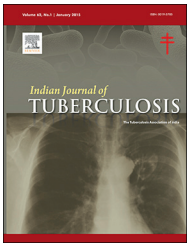

Original article

The impact of diabetics and smoking on gender differences of smear positive pulmonary tuberculosis incidence

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ABSTRACT

Background: Several determinants are responsible for different incidences of smear positive pulmonary tuberculosis (TB). The main determinants are cigarette smoking and diabetes mellitus. The aim of this study is to determine the effect of these risk factors effect modifiers in TB/gender association.

Methodology: In this retrospective cohort, relative risk of gender in developing smear positive TB as well as its interaction with smoking and diabetes mellitus were investigated.

Results: Of 1243 smear positive TB cases, 63.2% were male. Prevalences of diabetes mellitus among men and women TB cases were 9.2% and 22.9% respectively ($p < 0.001$). Frequency of cigarette smoking among men was significantly higher than that in women (61.7% vs. 7.6% respectively, $p < 0.001$). Male gender, increased the risk of developing smear positive TB as of 98% and 5% in smokers and non-smokers respectively ($p = 0.001$), indicating an interaction between gender and smoking in the effect on TB. In addition, male gender increased the risk of TB as of 13% and 34% in patients with and without diabetes mellitus respectively ($p = 0.300$) indicating no interaction between gender and diabetes mellitus on TB incidence.

Conclusion: Our study showed that cigarette smoking is a determinant factor for gender differences in TB incidence but diabetes mellitus does not affect the association between TB and gender.

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1. Introduction

Tuberculosis (TB) which is caused by Mycobacterium Tuberculosis is still one of the most important factors of morbidity and mortality worldwide.[1–3] According to the reports of World Bank, incidence of TB has increased from 160/100,000 in 1990, to 133/100,000 in 2014. The incidence has not changed in Afghanistan, while decreased from 32/100,000 to 22/100,000 in Iran.[4] In 2015, World Health Organization has reported the incidence of tuberculosis in Iran as of 16/100,000 which is more common among men.[5] However, some endemic areas in Iran have more than 100 TB patients per 100,000 populations.[1]

The difference of TB incidence reported between genders is not due to inequity and limited access to health services for women. Some investigations show that this difference is because of the biology and epidemiology of the disease.[6–10] However, in countries with more than 1% prevalence of HIV infection, the situation is completely different. For example, in Sahara in Africa, smear positive TB is more common among women due to higher prevalence of HIV infection among them.[11]

To determine the gender differences, it is important to consider the background factors may be associated with smear positive TB incidence. Previous studies have reported that HIV/AIDS, diabetes mellitus, cancers, malnutrition, cigarette smoking, alcohol addiction, homelessness, close contact with smear positive TB cases, imprisonment and poverty are the main risk factors for tuberculosis in developing countries.[12–15] Meanwhile, in some developing countries such as Iran, cigarette smoking and diabetes mellitus considerably increase the risk of developing tuberculosis.[16,17]

According to the available evidences, considering the factors responsible for gender differences in TB incidence is of great importance in the control of disease in the community. Investigating the electronic evidences showed that no studies have been carried out regarding the effect of diabetes mellitus and cigarette smoking in the gender differences of TB incidence. This study aims to assess the role of these factors in the incident of smear positive incidence among genders.

2. Materials and methods

This study is a retrospective cohort conducted in Mazandaran province located in northern Iran. Study samples were patients with smear positive and extra-pulmonary tuberculosis from districts of this province. Patients' characteristics have been registered into the CDC-TB registry in the TB control centers of these districts. After approval of the proposal in Mazandaran University of Medical Sciences, the required information were provided in Excel format without names.

All analyses were performed using Stata version 14 software (Stata Corporation, College Station, TX, USA). Categorical and continuous variables were described using percent frequency and mean (standard deviation) respectively. Patients' characteristics were compared between genders using Chi square test. The stratum specific relative risks for the associations between gender and TB were estimated bases on smoking status and diabetes mellitus. The test for heterogeneity was applied to detect the interaction between these variables and gender. p-value less than 0.05 was considered statistically significant.

3. Results

During the study period (2005–2013), 1243 new smear positive TB cases were identified 63.2% of them were male. Mean (SD), minimum and maximum age of them were 48.1 (20.), 11 and 98 years respectively. Mean (SD) age of men and women were 46.6 (19.8) and 50.6 (22.6) years respectively ($p = 0.001$).

Out of 1243 TB patients, 177 (14.2%) were diabetic. Prevalences of diabetes mellitus in men and women were 9.2% and 22.9% respectively ($p < 0.001$). In addition, 41.8% of patients were cigarette smoker. Frequency of smoking among men was significantly higher than that among women (61.7% vs. 7.6% respectively; $p < 0.001$) (Table 1).

Of 655 extra-pulmonary TB cases, 46.6% were male, 53 (8.1%) of which were diabetic. A significant difference was observed between men and women regarding prevalence of diabetes mellitus (4.9% vs. 10.9% respectively; $p = 0.004$). Frequency of cigarette smoking among these cases was 25.6% which was significantly higher among men than women (38.4% vs. 14.6% respectively; $p < 0.001$) (Table 2).

Prevalences of smear positive TB in patients under 15 and over 55 years were higher in women than men (2.8% vs. 0.4% respectively for <15 and 48.9% vs. 32.4% respectively for >55). On the other hand, men aged between 15 and 34 more than women developed smear positive tuberculosis. Frequency distribution of smear positive TB according to age group, area

Table 1 – Different characteristics of smear positive TB patients based on gender.

Variables	Male		Female		p-value
	Number	%	Number	%	
Age group					
<15	3	0.4	13	2.8	0.001>
15–34	288	36.7	139	30.3	
35–54	239	30.4	82	17.9	
≥55	785	32.4	458	48.9	
Prison					
No	571	72.7	433	94.5	0.001>
Yes	214	27.3	25	5.5	
Residence area					
Urban	460	58.6	264	57.6	0.393
Rural	325	41.4	194	42.4	
Diabetics					
No	713	90.8	353	77.1	0.001>
Yes	72	9.2	105	22.9	
Smoking					
No	301	38.3	423	92.4	0.001>
Yes	484	61.7	35	7.6	
HIV/AIDS					
No	89	11.3	40	8.7	0.011
Unknown	681	86.7	417	91	
Yes	15	1.9	1	0.2	
Total	785	100	458	100	–

Table 2 – Different characteristics of extra-pulmonary TB patients based on gender.

Variables	Male		Female		*p*-value
	Number	%	Number	%	
Age group					
<15	24	7.9	24	6.9	0.332
15–34	92	30.2	120	34.3	
35–54	90	29.5	113	32.3	
≥55	99	32.5	93	26.6	
Prison					
No	261	85.6	302	86.3	0.440
Yes	44	14.4	48	13.7	
Area residence					
Urban	176	57.7	222	63.4	0.078
Rural	129	42.3	128	36.6	
Diabetics					
No	290	95.1	312	89.1	0.004
Yes	15	4.9	38	10.9	
Smoking					
No	188	61.6	299	85.4	0.001>
Yes	117	38.4	51	14.6	
HIV/AIDS					
No	23	7.5	26	7.4	0.956
Unknown	280	91.8	321	91.7	
Yes	2	0.7	3	0.9	
Total	305	100	350	100	–

of residence, imprisonment and HIV/AIDS status are illustrated in Table 1.

According to the results of Table 3, male gender increased the risk of developing smear positive TB as of 98% and 5% among smokers and non-smokers respectively. These risk ratios were significantly different ($p = 0.0001$) indicating an interaction between smoking and gender on developing smear positive TB. In addition, male gender, caused 13% and 34% increase in the risk of smear positive TB among patients with and without diabetes mellitus. The difference in stratum specific relative risks was not statistically significant ($p = 0.300$) indicating no interaction between diabetes and gender in developing smear positive tuberculosis.

4. Discussion

Our study was carried out to investigate the effect of cigarette smoking and diabetes mellitus in the effect of gender on developing smear positive TB. The results showed that male-female ratio for smear positive TB, extra-pulmonary TB, concomitant presence of smear positive and extra-pulmonary TB, diabetes mellitus and extra-pulmonary TB, smoking and smear positive TB, smoking and extra-pulmonary TB were 1.7, 0.9, 0.7, 0.4, 13.8 and 2.3 respectively. The stratum specific relative risk estimates showed that male gender caused higher risk of developing smear positive TB among smokers than non-smokers. However, the effect of gender on developing TB was the same among diabetics and non-diabetics.

Cigarette smoking causes pathophysiological changes in respiratory system varies from local anatomical destruction to immunological problems. It weakens the immunity of the respiratory tract and also helps mycobacterial growth.[18,19] Prevalence of tuberculosis in diabetic patients is four folds of that in non-diabetics. Results of a study conducted in India predicted that the prevalence of diabetes mellitus will be increased from 20 to 30 millions in 2000 to 80 millions in 2030, thus a TB tsunami will be expected in parallel with the diabetes epidemics.[3,9,20] World Health Organization estimated the male-female ratio of smear positive TB cases as of 1.8.[21]

Mamaev et al. in 2008 showed that in diabetic patients, women have higher risk of developing tuberculosis.[22] The interaction between gender and diabetes was not mentioned in that study. Bacakoğlu et al. in 2001 did not find an interaction between diabetes and gender on developing TB. Low prevalence of diabetes mellitus could be an explanation for such variations, while in present, the aging in combination with diabetes may increase the effect of diabetes in developing smear positive TB.[23]

In a study conducted by Pérez-Guzmán et al.[24] among TB patients with and without diabetes mellitus, gender did not have a significant effect in developing pulmonary tuberculosis (51% men vs. 49% women). Singla et al. conducted another study in Saudi Arabia among TB patients with and without diabetes mellitus[25] and observed higher frequency of men in diabetic patients as compared with non-diabetics (two folds). Although previous studies did not directly mention the interaction effect of diabetes mellitus, they reported higher co-morbidity of TB and diabetes among men or vice versa.

Abal et al. showed that cigarette smoking did not have any effect on gender differences of TB which was in keeping with the results of the current study.[18] Similarly, Matsumoto et al. reported that TB-smoker men had higher prevalence of tuberculosis than women.[26] In addition, Watkins et al. showed that the differences in TB prevalence among men and women can be associated with smoking status which is similar to our results.[27]

Another study carried out in Hong Kong showed significant associations between TB and gender as well as cigarette smoking. It also revealed that these gender variations in TB might be due to the interactive effect of smoking.[28] Of 307 TB patients aged over 18 in India in 2012, 35.5% were diabetic, 9.8%

Table 3 – the association between gender and developing pulmonary TB according to smoking and diabetes mellitus.

Risk factors	RR for gender	*p*-value for gender	95% CI		*p*-value for interaction
Smoking					
Non-smokers	1.05	0.512	0.91	1.22	0.001
Smokers	1.98	<0.001	1.40	2.79	
Diabetes					
Non-diabetics	1.34	<0.001	1.18	1.52	0.300
Diabetics	1.13	0.434	0.83	1.52	

were smoker and 3.6% were diabetic and smoker.[29] It should be noted that the interaction between cigarette and diabetes with gender has not been considered in all of the above studies. However, it was the main goal of the current study. Therefore, such results are valuable.

One of the limitations of this study was the self reporting information regarding cigarette smoking. Another limitation was that the duration and number of cigarette smoked by patients had not been registered in the TB registry program.

In conclusion, our study provided evidences regarding the influence of cigarette smoking on the association between TB and gender. We also found that gender differences in TB prevalence was not associated with diabetes mellitus.

4.1. *Ethics and consent to participate*

This study has been registered in the Ethics Committee of Mazandaran University of Medical Sciences and supported by Research Deputy of this university. The Patients were informed in relation with the objective and the method of the study and they were accepted to consent in order to participate in the present study.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SA and MM carried out the design and coordination of the study and analysis. AN and MA participated in drafted the manuscript, data collection and the data entry. RT and MA helped to analysis the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors have none to declare.

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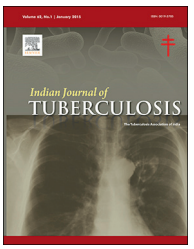

Original Article

A study of clinical profile of cases of MDR-TB and evaluation of challenges faced in initiation of second line Anti tuberculosis treatment for MDR-TB cases admitted in drug resistance tuberculosis center

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ABSTRACT

Objectives: To study the clinical profile of cases, evaluation of comorbidities and problems encountered in initiation of second-line drugs for multidrug-resistant tuberculosis (MDR-TB) patients.

Methodology: A prospective observational study was conducted on MDR patients admitted in drug resistance tuberculosis (DRTB) center of RDGMC Surasa Ujjain, a rural medical college, over a span of one year.

Results: Out of 130 admitted cases, majority (30%) were between 31 and 40 years of age. Males were predominant (70%). Females were significantly younger compared to males ($p = 0.00308$). Most patients (83.8%) were underweight (body mass index (BMI) < 18.5 kg/m^2). According to MDR-TB suspect criteria, majority were defaulter cases (39.23%). The anemia was the most common comorbidity (73.84%) among the study group followed by diabetes mellitus (9.23%), chronic obstructive pulmonary disease (COPD) (9.23%), 10 (7.69) asthma, 10 (7.69%) thyroid disease 9 (6.92%) followed by respiratory insufficiency 4 (3%), HIV 2 (1.5%), deep venous thrombosis (DVT) 2 (1.5%), renal failure 2 (1.5%), and hepatic failure 1 (0.76%). Majority had minimal lesion – 57 (43.8%), moderate – 38 (29.2%), and moderate advanced – 23 (17.7%) while far advanced was noted on X-rays in 12 (9.2%). A total of 91 (70%) cases had non-cavitary lesions and 39 (30%) had cavitary lesions, of which 27 were unilateral and 12 were bilateral.

Conclusion: The males were predominant in our study however females were affected at a younger age compared to the males. Most of the patients had taken Anti tuberculosis treatment (ATT) from Revised National Tuberculosis Control Program (RNTCP) in which defaulter and relapse were the major contributors of MDR-TB cases in our study. Radiological extent of lesions of these patients was less than expectation. Management of comorbidities is essential for compliance to treatment. It necessitates prolonged hospitalization and requires frequent follow-up in the DRTB center.

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1. Introduction

Tuberculosis is a major threat to world health, as it is the most common cause of death from an infectious disease worldwide.

Multidrug-resistant tuberculosis (MDR-TB) is caused by strains of Mycobacterium tuberculosis that are resistant in vitro to isoniazid (INH) and rifampicin with or without other anti-tubercular drugs based on drug sensitivity test (DST) results from a certified culture and drugs sensitivity laboratory. The treatment outcome results of MDR-TB patients remain suboptimal. In a meta-analysis, the cure rate for MDR-TB patients was reported at about 54–64%.[1]

There is a paucity of information on the pretreatment evaluation of the Indian MDR-TB patients. In this study, we present data regarding clinical characteristics of patients, the comorbidities detected on pretreatment evaluation, adverse drug reactions (ADR) associated on treatment initiation and thus the challenges faced in managing them in the Directly Observed Treatment Shortcourse (DOTS) plus site.

The WHO global resistance report (October 2013) estimated that there were 79,000 cases of MDR-TB from India in 2012.[2] This accounted for 17.6% of the world's MDR-TB burden. India and China accounted for nearly 50% of global MDR-TB. It is well known that poor treatment practices breed drug resistance. Areas with a poor TB control tend to have higher rates of drug-resistant TB. There is a lack of information on the management of MDR patients from the uncontrolled private sector.

2. Material and methods

A prospective observational study was carried out in drug resistance tuberculosis (DRTB) center covering the districts of Ujjain, Ratlam, Mandsaur, Shajapur, and Neemach. Approval from the institutional ethics committee was duly obtained. The study was carried out over a period of 12 month between January 1 and December 31, 2014. All the patients admitted in the center during this study period were enrolled in the study after obtaining informed written consent of patients or relative.

2.1. *Inclusion*

All patients of MDR-TB admitted in DOTS plus site.

2.2. *Exclusion*

Patients who did not complete one-week stay in hospital.

The diagnosed cases of MDR-TB are referred to the DRTB center with the referral form and DST report for initiation of MDR-TB regime. Pre-treatment evaluation was carried out after admission. It includes a detailed history, a thorough clinical examination, with investigations such as complete blood count, blood sugar to screen for diabetes mellitus, liver function tests, blood urea, thyroid function test, serum creatinine to assess the kidney function, and urine pregnancy test, and screening for HIV at Integrated Counseling and Testing Center and chest X-ray was done. Thereafter, patients were evaluated by expert committee and recommended to be put on MDR-TB regimen.

If any associated comorbidities were diagnosed or present previously, then the regimen was modified according to Programmatic Management of Drug Resistant TB (PMDT) guidelines. Every patient was admitted for a minimum period of seven days or it was extended as per requirement. Normally, patients were discharged after initiation of treatments with 7-day supply of drugs as extension pouches. Proper counseling regarding compliance to treatment, diet, infection control, and management of comorbidities/ADRs was given.

The severity of the lesion was classified into four groups on a chest X-ray using the classification of the National Tuberculosis and Respiratory Disease Association of the USA[3]:

- Minimal lesion.
- Moderate.
- Moderately advanced.
- Far advanced.

3. Observation and results

In our study, out of a total 130 MDR-TB patients, majority (30%) were in the age group 31–40 years (mean age = 36.80 years, standard deviation (SD) 13.52 years; range 13–72 years). The male-to-female ratio was 2.33:1. Mean age of females (29.63 $\pm$ 10.93 years) was less than that of males (41.83 $\pm$ 12.43). This difference was found to be statistically significant (Z-score = 2.96, $p = 0.00308$). Cough was the most common symptom occurring in 91.5% patients followed by loss of appetite. The occupational profile of patients revealed that a majority of them were laborers (50.80%) and farmers (30.80%) followed by housewives, students, etc. Among the patients, those who admitted to smoking were found to be 59 (45.4%) of total and 40 patients (30.8%) were also consuming alcohol on a regular basis. Most of the patients in our study were illiterate (60.80%) followed by education of primary school (32.30%), higher secondary (4.60%) and graduates (2.30%). The maximum number of patients were of lower class as per Kuppuswamy Scale, of which the upper lower class accounted for 52.3%, 45.4% were lower class, and only 2.3% were lower middle class. According to the MDR-TB suspect criteria, 51 (39.23%) were defaulters, 47 (36.15%) were relapse, 32 (24.61%) were failures, and only one patient (0.8%) was MDR contact.

Anemia was the most common comorbidity (73.84%) found among the study group followed by diabetes mellitus (12) (9.23%), chronic obstructive pulmonary disease (COPD), asthma, thyroid disease, respiratory insufficiency and corpulmonale, HIV, deep venous thrombosis (DVT), renal failure, and hepatic failure (as depicted in Table 1 and Fig. 1). A total of 109 patients (83.8%) were having a body mass index (BMI) less than 18.5 kg/m^2 in which 56.2% were severely undernourished with a BMI of less than 16 kg/m^2 followed by 20 (15.4%) with moderate thinness, and 16 (12.3%) were with mild thinness, 20 (15.4%) of patients had normal BMI, and only one patient was overweight.

Table 1 – Comorbidities associated with MDR-TB patients.

S. no.	Comorbidities	Frequency (*n*-130)	Percent (%)
1	Anemia	96	73.84
2	Bronchiectesis	31	23.84
3	Diabetes mellitus	12	9.23
4	COPD	12	9.23
5	Asthma	10	7.69
6	Thyroid disease (hyper + hypo)	9	6.92
7	Respiratory insufficiency and corpulmonale	4	3
8	HIV	2	1.5
9	DVT	2	1.5
10	Renal failure	2	1.5
11	Hepatic dysfunction	1	0.8

Hypo + hyper: hypothyroidism + hyperthyroidism; COPD: chronic obstructive pulmonary disease.

Pulmonary TB (PTB) was present in 128 (98.46%) patients, while extrapulmonary TB was noted in two (1.5%) patients (cervical lymph node and other scrofuloderma). Majority had minimal lesion in 57 (43.8%), moderate in 38 (29.2%), moderate advanced in 23 (17.7%) while far advanced was noted in 12 (9.2%). The maximum number of patients had taken Anti tuberculosis treatment (ATT) from Revised National Tuberculosis Control Program (RNTCP) (104 out of 130). Out of 130 patients, 115 (88.5%) patients had taken first-line drugs (FLDs) and 15 have been found to be taking second-line drugs (SLDs) from private sources.

4. Discussion

The emergence of resistance to FLDs and SLDs used to treat TB has become a significant public health problem in India and an obstacle to effective TB control. Areas with a poor TB control tend to have higher rates of drug-resistant TB. It has been acknowledged that good and effective initial treatment is a pre-requisite to the prevention of emergence of resistance. For this, it is important to study the patient characteristics and factors, which can contribute to resistance including addictions (alcoholism), education, economic status, etc.

In our study, covering populations mostly from the rural area, males constituted 70% (91 cases) while the females were 30% (39 cases) with male-to-female ratio 2.33–1. However, studies by Udwadia and Moharil[4] reported females were predominant which is based on urban area including well educated class of society.

Males are more at risk of exposure to the disease as they go out for work. The males being the breadwinners for the family are more likely also to get priority to seek medical care compared to the females. In the present study, majority of the MDR-TB cases (30.23%) were in the younger age group (31–40 years); mean age was 36.80 years. Udwadia and Moharil reported prevalence of younger age group among MDR-TB patients with the mean age of their study groups being 29.7 years and 33.25 years respectively. As most of our patients were from economically productive age group and some were the sole source of income for the family, the illness imposed an enormous economic burden. Impoverished and malnourished patient is more at risk to default and thus there is more risk of transmission of the disease to other family members.

In the present study, cough was most common symptom seen in 119 (i.e. 91.5%) patients. Other studies in India show that most common symptom was cough.[5]

Our patients had repeatedly taken many courses of ATT. The majority belonged to defaulter group, that is, 51 (39.23%), 47 (36.15%) cases were relapse followed by 32 (24.61%) cases of failure to previous anti-tuberculosis treatment. However, studies by Mukherjee et al. and Ünsal and Güler,[6] show a high relapse rate as predominant group. A study by Santha et al.[7] is similar to our study reporting defaulter as the most common group affected on the basis of previous ATT taken by patient. A very comprehensive education about the disease, treatment, early control of ADR, and comorbidities associated is very important for improved compliance.

In our study, out of 130 cases, 115 (88.5) had taken FLDs and 15 (11.5%) had taken SLDs. All the patients had taken second-line drugs from private source because all anti-tuberculosis drugs, both FLDs and SLDs, are widely available in India, and used, often liberally, in the private and public sectors outside of the RNTCP.

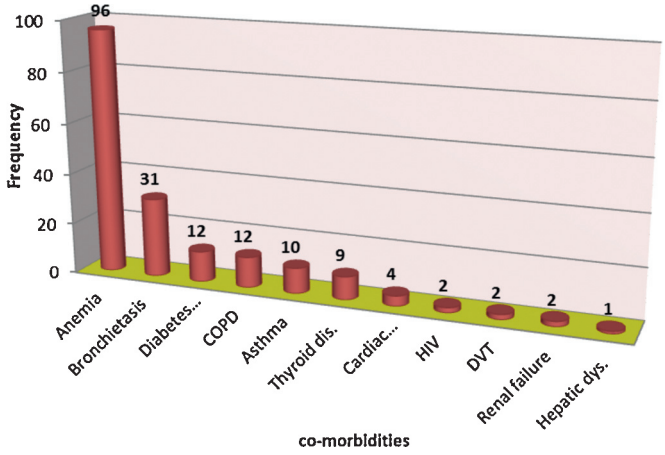

Fig. 1 – Different comorbidities associated with MDR-TB.

In our study, most of the patients had taken more than 1 episode of ATT, that is, 100 (76.92%) and only 30 (23.07%) patients had taken single episodes. Out of 15 who had taken second-line drugs, 13 had taken more than or equal to 3 episodes which is statistically highly significant (Chi-square value = 40.68 and *p*-value = 0.000).

In our study, most of the patients were from the rural area, that is, 67.7% of total and rest 32.3% resided in the urban area. Results were statistically significant (Z-score is 5.25 and *p*-value is 0.00). The study coincides with Elmahallawy et al.[8] who showed that 81.5% patients lived in rural areas and 18.5% of patient were from urban areas. Sobhya et al.[9] also reported that most of the patients were from rural areas.

The occupational profile of our patients revealed that a majority of them were laborers followed by farmer and housewives. Mukherjee et al. reveal that the most common group was household worker. Li et al.[10] show that unskilled workers are most commonly affected followed by farmer. A study by Atre et al.[11] also coincides with our study. Gupta et al.[12] observed a significantly higher prevalence of pulmonary TB in blue-collar than white-collar workers.

Based on Kuppuswamy Scale,[13] most of the patients in our study (68) (52.3%) belong to upper lower class followed by lower (59) (45.4%) and lower middle (32) (3%) class respectively. Better and higher socioeconomic groups of people are more likely to get themselves treated early and comply fully with the treatment.

In our study, most of the patients were illiterate (60.80%) followed by primary school (32.30%), higher secondary (4.60%) and graduate (2.30%). Khurram et al.[14] reported that 18 (60%) patients were illiterate in their study. Dholakia and Shah[15] reported 14.17% illiterate patients from an urban based study.

Our study reported significant number of patients in illiterate group because this is a rural-based study and in India, literacy rate was lower than other developed countries. Education is very important to know the exact duration, method of transmission of infection, curability, clinical symptoms, and prevention.

In our study, mean BMI was 16.02 ± 2.92 kg/m^2 (range 9.69–26.16 kg/m^2). 109 patients (83.8%) were undernourished with BMI less than 18.5 kg/m^2 in which 56.2% who were severely undernourished had BMI less than 16 kg/m^2 (Table 2).

The mean BMI of the present study was less than that of other studies because this is a rural based study and the majority of the patients belonged to lower socioeconomic strata with poor nutritional status.

It means that nutritional supplementation may represent a novel approach for fast recovery in tuberculosis patients. In addition to raising nutritional status of the population, it may prove to be an effective measure to control tuberculosis in the underdeveloped areas of world. Immunity plays an important role in control of the disease and better nutritional status and guidance on proper dietary intake during hospitalization can go a long way in control of disease.

Table 2 – Mean body mass index (BMI) was 18.67 ± 3.45 (range, 14–23.5).

S. no.	Different studies	Mean BMI (kg/m^2)	Range
1	Vishakha et al.[16]	18.67 ± 3.45	14–23.5
2	Mukherjee et al.[5]	18.5 ± 4.025	12–31
3	Present study	16.02 ± 2.92	9.69–26.16

In our study, the most common comorbidity is anemia contributing towards 96 (73.84%) patients. A total 96 patients were anemic in which moderate anemia was most commonly seen in 53 (40.2%) followed by mild in 26 (19.7%), severe in 10 (7.6%), and life-threatening anemia was seen in 7 (5.3%). Most of the patients of our study were of low socioeconomic status and suffering from malnutrition, so that anemia is the most common comorbidity present in our study requiring blood transfusions in severe cases and extending the duration of hospitalization.

In our study, one patient was found to be pregnant (less than 20 weeks duration) on pretreatment evaluation. Therefore, the patient was counseled, and the medical termination of pregnancy was carried out in view of the potential risk to both the mother and fetus. Thereafter, the patient started on MDR regimen.

Bronchiectasis: Caseation necrosis and granulomatous inflammation in the lung lead to extensive lung destruction and bronchiectasis. This is the second most common comorbidity found in our study after anemia. Bronchiectasis is the major comorbidity leading to secondary infection in patients requiring prolonged admission, in certain cases higher antibiotics and subsequently further loss of lung reserve.

In our study, diabetes was the third commonest comorbidity, that is, 12 out of 130 (9.23%) on pretreatment evaluation. Some of the patients had to be shifted on insulin therapy from oral hypoglycemic agents. It has been seen that diabetics have a higher incidence of anti-tuberculosis drug resistance. Diabetes patients might also have some degree of impaired gastro-intestinal drug absorption even in the absence of clinical gastro-paresis. The results of this study have been challenged.

Singla et al.[17]: The study revealed that PTB patients with associated diabetes have a similar clinical presentation, a higher bacillary load at the time of diagnosis of PTB, and a lower prevalence of anti-tuberculosis drug resistance as compared to the studies by Ibrahim and Sobhy as shown in the table below.

Number	Study	Cases with DM	Percentage
1	Elmahallawy et al.	30/200	15%
2	Sobhy et al.	3/29	10.3%
3	Kikvidze and Ikiashvili[18]	104/1970	5.8%
4	Present study	12/130	9.23%

In our study, 12 out of 130 (9.23%) were suffering from COPD. Kapadia et al. study shows that COPD is the common comorbidity. Poulomi et al. reported most common comorbidity among study group (17.44%). COPD too contributes to the late detection of TB and some of the patients have poor lung functions, even respiratory failure requiring domiciliary oxygen therapy. As most of the patients are unable to afford it, this again prolongs hospitalization.

Hypothyroidism is a known side effect of thioamides – ethionamide (ETH), prothionamide, and para-aminosalicylic

acid.[19–22] Hypothyroidism has vague and non-specific symptoms, which can be easily missed in our study. 8 out of 130 (6.15%) patients were suffering from hypothyroidism. All patients were treated with thyroxin. The dosage of thyroxin was adjusted based on clinical status and laboratory results at the DRTB center facility. Among all eight patients, no one was required to stop or replace ETH with reserve drugs. Kapadia et al. did not report any patient with thyroid abnormality.

In our study, four patients (3.07%) were having destroyed lung with respiratory failure leading to corpulmonale with pulmonary arterial hypertension and congestive cardiac failure. All of the patients had cardiac abnormality secondary to lung condition. Ibrahim et al. reported 1.5% of cardiac disease in their study. Duraisamy et al.[23] reported 2.8% of patients having cardiac comorbidities; this study coincides with our study.

In our study, only 2 (1.5%) cases were HIV seropositive. They were already on anti-retroviral drugs (ART) and one patient was having hypothyroidism.

Renal insufficiency can be due to renal TB, previous use of aminoglycosides or concurrent renal disease. Great care should be taken in the administration of second-line drugs in patients with renal impairment. In the present study, two cases of renal failure were reported. We started them on modified MDR-TB regimen based on glomerular filtration rate. The patients were advised regular follow-up.

In our study, 2 (1.5%) cases of DVT were diagnosed and were managed with heparin sulfate according to body weight. They required prolonged hospitalization.

One patient was having deranged liver function on pretreatment evaluation. In that case, pyrazinamide was temporarily withheld with close monitoring of liver function. After liver function had normalized, the patient was restarted with pyrazinamide.

Some common ADRs observed on initiation of second-line drugs were nausea, vomiting, joint pain, and itching. They were managed symptomatically and treatment had to be modified in some cases with deranged renal and liver functions. However, it is not only the pill load and resultant side effects that have to be managed but also the underlying COPD, anemia, DM, renal failure, etc. which have to be effectively managed to prevent patient from defaulting in their treatment.

The importance of educating the patient and the attendants about the disease and medications during the hospitalization too is very important to ensure compliance to treatment.

5. Conclusion

Majority of our patients were from rural areas with lower socioeconomic status and low level of education, which are important risk factors for MDR-TB.

There is need to further educate the population as well as health providers in the symptomatology of tuberculosis for the early diagnosis of TB as defaulters contribute greatly to drug resistance.

The most common symptom was cough, followed by loss of appetite, shortness of breath, fever, chest pain, and hemoptysis. So it is important to educate community and health care provider about symptomatology for early diagnosis and treatment.

Most of the patients had taken ATT from RNTCP in which defaulter and relapse were major contributors of MDR-TB suspect in our study. There is need to further enhance performance of the program and educate the DOT provider so that they can assess side effects early and manage them effectively to prevent drug default. They should be able to refer serious adverse effects to higher center.

It was seen in our study that comorbidities were associated with MDR patients including anemia, diabetes, COPD, asthma, renal failure, DVT, etc. The identification and effective management of these comorbidities is very important in the management of MDR-TB cases. This also helps in preventing drug default in the patients.

As the anemia is the most common comorbidity, 7.6% and 5.3% of patients were suffering from severe and life-threatening anemia, respectively, and this had to be treated by giving blood transfusion while moderate to mild anemia were given oral and parental iron supplementation.

Other comorbidities such as DVT and renal failure was treated according to PMDT guideline; however, they required prolonged hospitalization.

MDR-TB should be managed very effectively with careful use of second-line drugs to reduce morbidity and mortality and to prevent its further transmission and the development of Extensively Drug Resistant TB (XDR-TB).

Conflicts of interest

The authors have none to declare.

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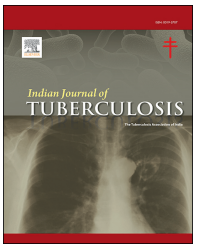

Original article

Impact of pharmacist counseling and leaflet on the adherence of pulmonary tuberculosis patients in lungs hospital in Indonesia

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ABSTRACT

Background: One of the goals of counseling in patients with chronic diseases including tuberculosis patients is to improve adherence to taking medication. By patient adherence, therapeutic results are more optimal. Additional counseling alternatives such as leaflets may be needed to make easier for patients to obtain information about their treatment. This study aimed to analyze the effectiveness of counseling with and without leaflets on the adherence on taking tuberculosis (TB) drugs.

Methods: This study was a quantitative research conducted using a quasi-experiment method with a control group for pre-test and post-test design. Data was taken by consecutive sampling. The number of samples in this study was 75 respondents which divided into three groups: counseling, counseling with leaflets, and control that is a usual care in hospital. The inclusion criteria were patients diagnosed with pulmonary tuberculosis with age 25–55 years, who has been taking TB medicines for at least one month and can communicate well. Data was analyzed using Wilcoxon and Kruskal–Wallis with post hoc Mann–Whitney due to abnormality of the distributed data.

Results: Before the intervention, of 20 respondents (42.6%) out of 75 respondents were obedient to their TB medicines, whereas after the intervention the number of obedient patients was 33 respondents (70.2%). There was a significant increase in adherence between before and after two weeks of counseling intervention with a p-value of 0.029 before and after two weeks of counseling with leaflets with a p-value of 0.003. Counseling and counseling with leaflets improved patients' adherence compare to control group with p-values of 0.028 and 0.001 respectively.

Conclusion: Counseling and counseling with leaflet impact in patients' adherence to tuberculosis medication.

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1. Introduction

Pulmonary tuberculosis (TB) is one of the infectious diseases which is a public health problem in the world. TB is one of the top ten cases of death worldwide. In 2017, 1.6 million died from this disease including 0.3 million among people with HIV. TB is leading killer of HIV patients.[1] Indonesia has the second highest burden in the world which the number of TB cases is estimated to have 1 million TB cases per year. Around 7500 of the notified cases die and probably there are more than 100,000 deaths every year among those not notified.[2]

Tuberculosis is caused by bacteria that call *Mycobacterium tuberculosis*. This bacterium most affect lungs.[1] Treatment of TB disease is carried out for at least six months and is given in two phases.[3,4] To achieve healing is very important for patients with pulmonary TB to have knowledge of the disease. This knowledge is in terms of regularity, completeness, and compliance in taking TB medicines. Non-adherence of patients to treatment is a major risk factor that causes multidrug resistance tuberculosis (MDR-TB). Non-adherence increases MDR risk 7.75 times compared to obedient patients.[5]

Based on the results of a previous study of 80 respondents in the lung hospital in Surakarta, it was found that there were 30 respondents who were not compliant in the treatment of pulmonary tuberculosis (37.5%).[6] Although the number of non-compliance patients is 37.5% there is a greater likelihood that there will be a resistance problem in therapy. Treatment of tuberculosis resistance is more difficult, expensive, and a relatively low cure rate.[1,7] The impact of MDR is not only in the difficulty on treatment but also its impact on economic, social and psychological.[8,9] Disobedience of pulmonary TB patients on TB treatment can be caused by several factors including lack of knowledge, lack of social support, a side effect of TB drugs, believing about medicines, and forgetfulness.[6,10,11]

For improving better treatment adherence, comprehensive health education, especially for patients at treatment sites, need to be addressed. Pharmacist counseling lead to improving patients adherence.[12–16] Additional media such as leaflet may be needed to make it easier for patients to get the information to be related to the treatment being undertaken. Patients can read leaflet about their medication anywhere and anytime and it may minimize their forgetfulness about their TB medication and improve their knowledge. Improving patient knowledge can increase patients' awareness of the disease and the risk of complications so that patients become obedient. Although there have been several studies related to the influence of counseling on adherence in some countries, but research on the effect of counseling and leaflets on TB patient compliance has never been done in Indonesia. Further, it is inappropriate to extrapolate the finding of counseling effect conducted in different disease and other countries to the local situation because of differences cultures. Therefore, it is necessary to study the effect of counseling and leaflets on the compliance of pulmonary TB patients in their treatment at the Community Lung Health Center in Surakarta Indonesia.

2. Methods

2.1. Study setting

This study was conducted in a lung hospital in Surakarta Indonesia. This hospital is a referral hospital especially in lung disease in Central Java Province, Indonesia.

2.2. Study design and sampling

This research was a quantitative study with a research design using quasi-experimental method with pre-test and post-test control group design. The sampling method was conducted by convenience sampling. The focus of this study was examined the level of compliance, the effect of counseling and counseling with leaflets on patient adherence to TB treatment compared to the control group. The independent variable of this study was counseling and leaflets. The dependent variable was the adherence in TB medicines.

2.3. Ethical consideration

Approval of the study was obtained from Health Research Ethics committee, Faculty of Medicine of Universitas Muhammadiyah Surakarta No. 1084/C.1/KEPK-FKUMS/II/2018 before the commencement of the study. Informed concerned was done and participants' confidentiality was maintained during and after collecting data.

2.4. Tool

The adherence questionnaire contains 13 questions or items. The adherence questionnaire was reviewed by two academics in clinical pharmacy background and community pharmacy and a physician in infectious disease, while the leaflet was reviewed by two pharmacists and a physician in infectious disease. After reviewed by panel experts, adherence questionnaire was pretested on 30 respondents as a pilot study to clarify any ambiguities. The validation and reliability of the questionnaire was then analyzed statistically with Pearson Correlation and Cronbach Alfa. The questionnaire is valid if r-count is more than r-table or p-value <0.05 and reliable if Cronbach alfa more than 0.6.[17]

2.5. Data collection

The primary data was collected from the patients by interview method and the secondary data was taken from patient medical records. Subjects were 75 outpatients with pulmonary tuberculosis at the Center for Lung Health, Surakarta, divided into three groups: patients received counseling, patients received counseling with leaflets, and patients with neither counseling nor leaflet intervention. The inclusion criteria were:

a. Patients diagnosed with pulmonary tuberculosis at Lung Hospital Surakarta.
b. Adult patients aged 25–55 years because they are able to make their own decisions.

c. Tuberculosis patient outpatient who have undergone TB medicine for at least 1 month.
d. Willing to be a respondent, follow research procedures
e. Be able to communicate.

The exclusion criteria was TB patients who pregnant. The steps of collecting data can be seen in Fig. 1.

2.6. *Data analysis*

Data was analyzed by Kolmogorov-Smirnoff. If p-value <0.05 the data was not in normal distribution and vice versa. In this study data was not normally distributed, non-parametric analyses were approached. Wilcoxon analysis was used for groups of paired data which was analyzing the relationship between before and after intervention (pre-test and post-test). While the Kruskal–Wallis post hoc Mann–Whitney analysis was used for groups of unpaired data, namely analyzing differences between intervention groups and the control group.

3. Result

Before the adherence questionnaire was used, it has been validated and this questionnaire was valid and reliable to use as a tool to know the patients' adherence. Of 30 respondents for the validity test, the r table is 0.301. The question is valid if the r > 0.301. The result of r > 0.301 so that all the questions in the adherence questionnaire are valid. The result can be seen in Table 1. This questionnaire also reliable because Cronbach alfa was 0.686 > 0.6. Adherence questionnaire consists of 13 items with yes or no answers. Items number 2, 4, 13 each score 1 if the answer is "no", while items number 1, 3, 5, 6, 7, 8, 9, 10, 11, 12 each score 1 if the answer is "yes". The total score is 13.

During the period of study on July–December 2017, 75 patients were recruited as respondents. Respondents divided by three groups each group consist of 25 patients. First group was intervention for counseling and leaflet, the second group was intervention for counseling, and the third group was control group. The characteristic of respondents is presented in Table 2, level of patients' compliance to take TB medicine before and after intervention is in Table 3, and result of Kruskal–Wallis post hoc Mann Whitney Test for Comparison of intervention and control on medication adherence can be seen in Table 4.

4. Discussion

In this study, the number of male patients (54.7%) is more than females (45.3%). This result was similar as the other researches.[5,18,19] A man has a higher risk to be infected tuberculosis because of differences in social roles, risk behaviors, and activities. Males may travel more frequently; have more social contacts; spend more time in settings that may be conducive to TB transmission. On the other hand, women have more-robust immune responses to infection.[20] Of the 48 respondents, (64%) were underweight. In this study showed that the most TB patients at the age of 25–45 years amounted to 56 respondents (74.7%) while the age of 46–55 years amounted to 19 respondents (25.3%). This result was in agreement with the other studies. All age groups are at risk of tuberculosis infection, but TB disease mostly affects adults in their most productive years.[1]

The education level category in this study shows that junior and senior high school education is higher with the incidence of pulmonary TB compared to higher education. Junior high school education level was 22 respondents (29.4%), a senior high school with 36 respondents (48%) while with a higher education level only 6 respondents (8%). Level education significantly associated with a higher risk of TB[21] and educational status were significantly associated with knowledge level.[22]

Based on Table 3, in the intervention group of counseling with leaflets, before the intervention, non-compliance patients were 14 patients (56%) and after the intervention, the number of non-adherence patients significantly decreased to

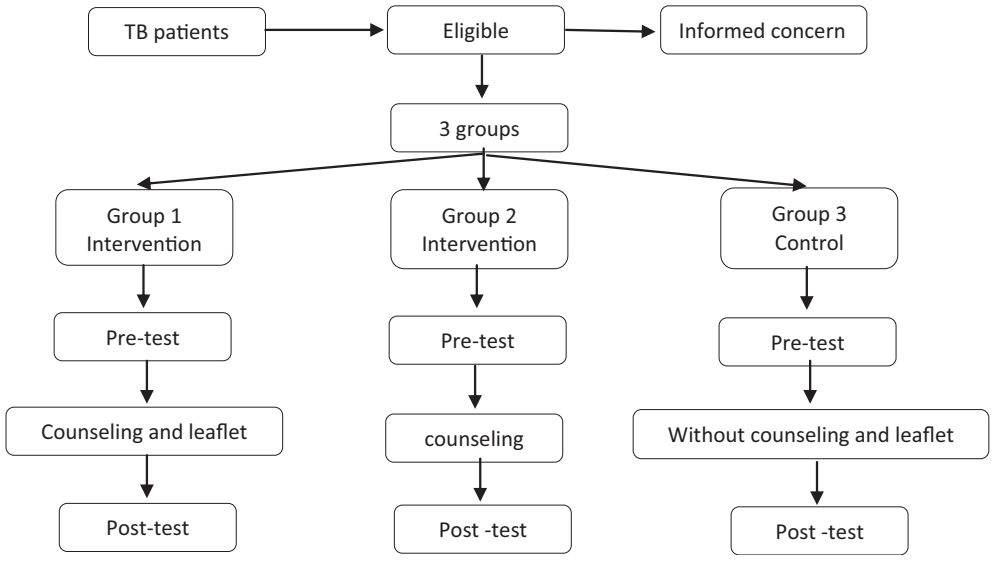

Fig. 1 – The step of collecting data.

Table 1 – Test of validation of adherence questionnaire.

Item	Questions	r	note
1	Have you ever forgotten to take TB medication?	0.464	Valid
2	Do you take TB medication on time and regularly every day?	0.375	Valid
3	Have you ever reduced or overestimated the amount of medicinal from the amount you should drink?	0.527	Valid
4.	Did you yesterday/the day after taking TB medicine?	0.412	Valid
5	Have you ever forgotten to take TB medicine when traveling or leave home, so you don't take medicine?	0.474	Valid
6.	When you feel better, have you ever stopped take TB medicine?	0.412	Valid
7	When you feel worsen, have you ever stopped drinking TB medicines?	0.375	Valid
8	Have you ever taken TB medication not according to the recommended frequency?	0.441	Valid
9	Have you ever in intentional not taken TB medicine?	0.469	Valid
10	Have you ever discarded pulmonary TB medicine?	0.476	Valid
11	Have you ever replaced TB medicine given by a doctor with herbal medicine?	0.377	Valid
12	Do you take TB medicine only when you feel sick?	0.516	Valid
13	Do you immediately come for treatment to take the medicine before the medicine runs out?	0.691	Valid

Table 2 – Patients' demographics.

No	Demographics	Number	Percentage
1	Gender		
	Male	41	54.7
	Female	34	45.3
2	BMI		
	Underweight	48	64
	Normal	23	30.7
	Overweight	4	5.3
3	Age		
	25–45	56	74.7
	46–55	19	25.3
4	Occupation		
	Private Employees/Employees	37	49.3
	Entrepreneur	18	24
	Housewife	11	14.7
	Others	9	12
5	Education		
	No school	1	1.3
	Elementary school	10	13.3
	Junior School	22	29.4
	Senior high school	36	48
	Diploma	6	8
6	Treatment phase		
	Intensive phase	35	46.7
	Continuation phase	31	41.3
	Category 2	9	12
7	Type of medicine		
	FDC	69	92
	Anti-TB drugs	6	8

BMI=Body Mass Index; FDC=Fixed-Dose Combination.

4 respondents (16%) with p-value 0.003. On the other word, the number of adherence patients increased more than two times before and 2 weeks after the intervention of counseling and leaflet in 11 patients (44%) to 21 patients (84%). The significant increase of compliance patients was the same as a group of counseling intervention with p-value was 0.029. In pre-test, 9 patients (36%) were compliance with their TB medicines and 14 patients (56%) were adhere in 2 weeks of post-test. There is influence statistically significant giving counseling and leaflets or counseling to the level of compliance of pulmonary TB patients in taking TB medicine before and 2 weeks after intervention. On the other hand, in the control group, the number of patients who adhered to treatment at the time of post-test decreased from 19 patients (40%) to only 6 patients (24%).

In Table 4 result of Kruskal–Wallis test shows that p-value is 0.001. It means that there is a statistically difference among groups. Post hoc test with Mann–Whitney is needed to compare among groups and this test shows there is a significant difference on interventions between counseling with leaflets group compare to control group on the level of adherence to taking TB medication for pulmonary TB patients because the p-value is $0.001 < 0.05$. Giving counseling with leaflets has an influence on increasing TB patient compliance in taking medicine. Similarly, there is a significant difference in the impact of giving a counseling intervention to control group on the level of adherence to taking TB medicine for pulmonary TB patients because P value is $0.028 < 0.05$. Interventions between counseling with leaflets compare to counseling interventions on the level of adherence is statistically significant difference as well with p-value 0.026.

Regarding this issue, similar results obtained from the study in Tehran and New Jersey in the year 2016 and 2014 respectively which shows counseling patients improves patient's medication adherence and treatment satisfaction and consequently improves clinical outcomes. Counseling activity was beneficial in helping patients understand their medication.[12,23] There are many advantages of pharmacist counseling can be obtained: ensure the safety and effectiveness of treatment, patients get additional explanations about the disease, helps solve therapeutic problems in certain situations, can reduce drug use errors, improve compliance in carrying out therapy, avoid unwanted drug reactions, and improve the effectiveness & efficiency of health costs.[24] In a systematic review revealed that pharmacist education has a positive impact on the adherence and completion of prescribed tuberculosis medications.[25] Medication counseling is

Table 3 – Level of patient compliance in taking medicine before and after the intervention.

No	Groups	Pre-test		Post-test		P value
		Number	Percentage	Number	Percentage	
1	Counseling and Leaflet					
	Adherence	11	44	21	84	0.003
	Non-adherence	14	56	4	16	
2	Counseling					
	Adherence	9	36	14	56	0.029
	Non-adherence	16	64	11	44	
3	Control					
	Adherence	10	40	6	24	0.537
	Non-adherence	15	60	19	76	

Table 4 – Kruskal–Wallis Test Results Comparison of Counseling and counseling with leaflets and control on medication adherence level.

No	Groups	Number	Adherence	P value
1	Counseling and Leaflet	25	13 (12–13)	<0.001
2	Counseling	25	13 (10–13)	
3	Control	25	12 (10–13)	

Kruskal–Wallis test. Test of post hoc Mann–Whitney: counseling and leaflet versus counseling p = 0.026; counseling and leaflet versus control p = 0.001; counseling versus control p = 0.028. The data was in median (minimum–maximum).

one of the key interventions to enhance patients' knowledge and understanding of their medications and how and why to take them, hence improving adherence.[26]

Alternative media additional counseling is needed to make it easier for patients to get the information needed to be related to the treatment being undertaken. One alternative is the use of leaflets so that patients can read them wherever and whenever. Leaflets can help patients improve self-efficacy and self-management. Improving patient knowledge can increase patient awareness about the disease and risk of complications so that patients become obedient. Similar to a study performed by Kayalli in 2014, which was done on 486 patients, patients wanted to receive information orally and in writing. More than half of the patients (60%) read the leaflet and found it a useful source.

5. Conclusion

Compliance with pulmonary TB patients in taking TB medicine needs to be improved by Since counseling give a positive impact on enhancing patient's compliance to take TB medicine, it presents an opportunity for pharmacists to provide clinical services that utilize pharmacist strength and ensure positive therapeutic outcomes for patients. One of the pharmacist services is providing counseling about TB medicine. Additional media such as leaflets to improve patient compliance in taking medicine can be considered. Leaflets help patients to be obedient because they can be read by patients wherever and whenever. Leaflets significantly improve patient knowledge. Increased knowledge of patients can increase patient awareness about the disease and the risk of complications so that patients become obedient in taking TB medicine. Improving adherence is not only patient's responsibility, but also it is needed to good cooperation among the government, health professionals, community and patients' family to achieve therapeutic goals, minimize of TB transmission to the other people, and prevent the occurrence of bacterial resistance or multidrug resistance.

Conflicts of interest

All authors have none to declare.

Author contribution

Hidayah Karuniawati
- ✓Had the idea of this study and designed the proposal.
- ✓Developed the protocol.
- ✓Read and approved the manuscript.

Author contribution

Okta Nama Putra
- ✓Took data from medical record.
- ✓Interviewed patients and gave intervention.
- ✓Entry data and analysis.
- ✓Read and approved the manuscript.

Author contribution

Erindyah Retno wikantyasning
- ✓Entry data and analysis.
- ✓Read and approved the 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.2019.02.015.

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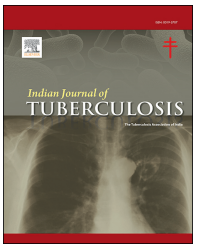

Original Article

Endo-bronchial application of glue in the management of hemoptysis

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ABSTRACT

Background: Hemoptysis from varied etiologies, often fails to respond to conservative therapy. The conventional managements of such a situation are Bronchial Artery Embolization (BAE) or thoracic surgery which is often not possible. Endoscopic application of glue may stand as a method of therapy in these circumstances.

Methods: 202 patients of hemoptysis were treated by video-bronchoscopy assisted endobronchial application of glue (n-butyl cyanoacrylate) with the help of polyethylene catheter being placed through the working channel. The details of the procedure and their 6 month follow up are presented.

Results: Immediate control of hemoptysis was achieved in 183 i.e. 90.59% of patients. 19 patients had a partial response, i.e., hemoptysis stopped and then recurred, endobronchial application of glue was repeated in them out of which 14 (6.9%) responded to the second procedure whereas 5 (2.47%) failed to show any response in spite of the repeated procedure. The complication rate was 0.49% in the form of glue migrating into the trachea. There was no mortality.

Conclusion: Endobronchial application of glue for hemoptysis can be an effective, economic and alternative therapy for mild to moderate hemoptysis.

1. Introduction

Hemoptysis is defined as the spitting up of blood derived from the lungs or bronchial tubes as a result of pulmonary or bronchial hemorrhage.[1] We have a large base of patients with hemoptysis from various etiologies who do not respond to conservative management including use of cough suppressants, antibiotics, haemostatics & anxiolytics. Medical glue, N-butyl cyanoacrylate has been used successfully in humans by various medical specialties without any side effects.[2,3] We attempted an inexpensive, effective and safe alternative in the form of endobronchial placement of glue (n-butyl cyanoacrylate) under direct vision with the help of therapeutic video bronchoscope (OLYMPUS T-180) to control the hemoptysis.

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2. Materials and methods

All patients registered between years 2004 and 2015 with mild, moderate and even severe cases of hemoptysis were included. Mild, moderate and severe hemoptysis were defined as streaking to less than 30 ml bleeding, 30–200 ml and >200 ml of blood per given 24 hours, respectively.

2.1. *Inclusion criteria*

For consideration of the glue therapy, patient should be hemodynamically stable and not in need of any blood transfusion or life-support system. In mild and moderate cases, we tried conservative treatment for 7 days. We have done the procedure even in severe cases as first line life saving management.

2.2. *Exclusion criteria*

Patients with serious co-morbidity like congestive heart failure, chronic renal failure, poor cardio-respiratory reserve, advanced age with neurological deficit and poorly controlled diabetes mellitus or hypertension were excluded. We also did not consider endobronchial application of glue therapy when we were unable to localize the source of bleeding and/or if the blood was seen coming out from multiple segments. Overall, we studied 202 patients with hemoptysis.

2.3. *Evaluation*

Proper informed consents were obtained in all the patients and the following investigations hemoglobin, TLC, DLC, ESR, APTT, PT, BT, CT, chest x-ray and sputum for AFB were done.

2.4. *Procedure*

The bronchoscopies were performed in accordance with American Thoracic Society guidelines for bronchoscopy.[4] Total dry conditions were ensured by the bronchoscopist & the technician, which meant that the polyethylene catheter, syringes filled with air and those syringes filled with glue were kept away from any fluids. Acetone was kept ready in a covered sterile bowl. A 0.5 ml vial of Glue (N-butyl-cyanoacrylate) was opened & filled in the syringe. The bleeding sites were localized by direct visualization or confirmed after washing with saline. Polyethylene catheter was passed through the working channel of the bronchoscope & placed at the bleeding segment or embedded in to the clot (Fig. 1). Bronchoscope was withdrawn 4–5 cm from the treatment site where the glue was needed to be injected but keeping the catheter in place & under vision. If the bleeding did not stop with the first instillation, additional 0.5 ml of glue was injected in the same segment. Glue was injected maintaining the dry conditions (Fig. 2) and once this was carried out the whole assembly i.e. bronchoscope & the catheter were removed simultaneously and immediately. The catheter was cut at the proximal end and withdrawn from the distal end of the bronchoscope. The external surface and the working channel of the bronchoscope were cleaned with acetone if there was any spillage of the glue. Repeat bronchoscopy was performed immediately to confirm the proper placement of the glue (Fig. 3). Once glue was seen in place and there was stoppage of the bleeding the procedure was labeled as successful. Repeat chest x-ray was performed 24 hours later to rule out any segmental or sub-segmental collapse. Patients were followed up with chest x-rays and bronchoscopies at one, three and six months.

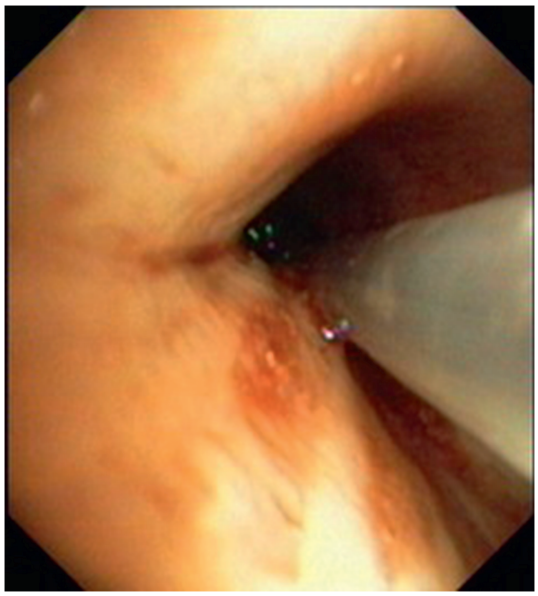

Fig. 1 – Catheter over the site of hemoptysis.

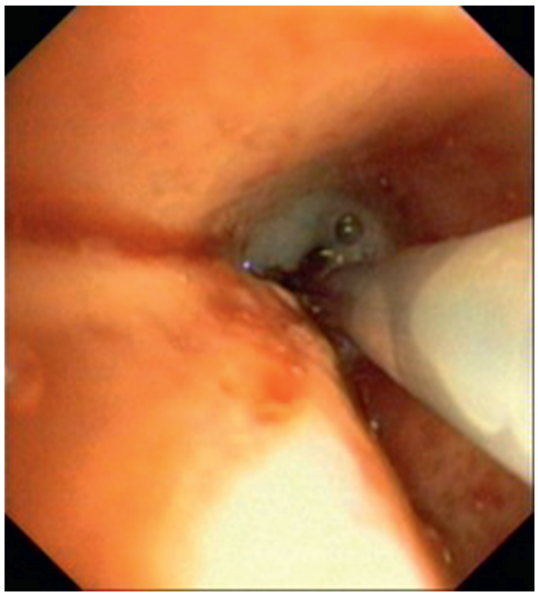

Fig. 2 – Glue being injected at the Site of Hemoptysis.

3. Results

A total of 202 patients were studied, 128 (63.36%) were males and 74 (36.64%) females. 116 of them (57.42%) presented with a normal chest x-ray (74 males and 42 females) whereas 86

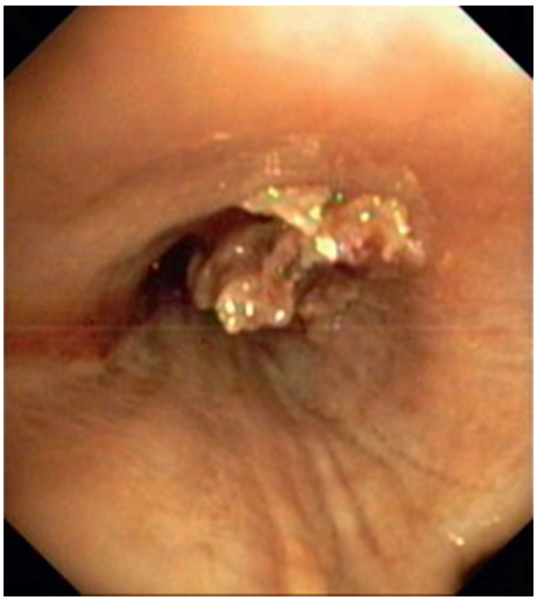

Fig. 3 – Dried glue immediately after instillation.

(42.58%) had abnormal radiological findings. Other demographic data are given in Tables 1–4.

The abnormalities were mainly in upper zones in 89 patients, but other areas were also involved. The source of bleeding was in main airways in majority of patients. Clot was visualized in either the right main bronchus or bronchus intermedius or left main bronchus in 81 (40.1%) of cases and was treated in situ. In 37 (18.31%) of cases bleeding was from the right upper lobe bronchus and in 26 (12.87%) the source was from right middle lobe. The details are given in Table 5.

Hemoptysis was immediately controlled in 183 of the 202 (90.59%) patients. However, repeat bronchoscopy with glue therapy was required in 19 cases out of which it was not possible to stop the bleed in 5 (2.47%). On repeat bronchoscopy the glue was seen as a shiny white substance (Fig. 4). Seventy six (37.62%) patients expectorated out the glue within a few days to 3 months. Patients were followed up with chest x-rays and bronchoscopies at one month, three months and six months. Migration of the glue was observed in 29 (14.35%) of cases. No recurrence of hemoptysis was observed in a follow up of 6 months (except for 5 initial failures in spite of the repeated procedure). One patient had a complication where the patient developed spasm and feeling of something sticky in his throat just after the procedure. Immediate check bronchoscopy was done which revealed the glue migrated to and partially obstructed the trachea. It was removed with the forceps and the symptoms were resolved (Fig. 5).

In 16 patients (7.92%) the glue was immediately expectorated post-procedure on the table with bouts of cough (Fig. 6) and the procedure was repeated. Glue injected in clots was very successful in 121 (59.9%) of cases. Procedure was considered as a failure when fresh blood continued to be expectorated out along with the sputum. The patients were followed up with X-rays and bronchoscopic examination at one month, 3 months and 6 months. In 126 (62.37%) of cases, glue was still in place, but in others the bronchii were clear on follow up bronchoscopic examination. Only 3 patients had the glue still in place at the end of 6 months.

Table 1 – Etiologies of hemoptysis.

Etiologies Of Hemoptysis	No. of patients
Pulmonary Tuberculosis	127
Lung Malignancies	19
Bronchiectasis	29
Actinomycosis	3
Aspergillosis	3
Tropical Pulmonary Eosinophilia	2
Idiopathic Thrombocytopenic Purpura	2
Destroyed Lung	17
Total	202

Table 2 – Age wise distribution of the patients.

Age	No. of patients
15–24	14
25–34	37
35–44	67
45–54	60
55–64	24

Table 3 – Radiographic Site of Lesions (Many of the patients had bilateral findings, so the total is not mutually inclusive).

Zones involved	No. of patients
Normal Chest X-Ray	73
RUZ	55
RMZ	35
RLZ	16
LUZ	34
LMZ	13
LLZ	13

Table 4 – Duration of hemoptysis.

Duration of Hemoptysis	No. of patients
7–15 days	38
16–30 days	67
1 month–2 months	41
2 months–3 months	24
>3 months	15
Severe Hemoptysis	17

Table 5 – Site of hemoptysis.

Site of Hemoptysis	No. of patients
Right Main Bronchus	35
Bronchus Intermedius	19
RUL	37
RML	26
RLL	13
Left Main Bronchus	27
LUL	23
Lingula	14
LLL	8

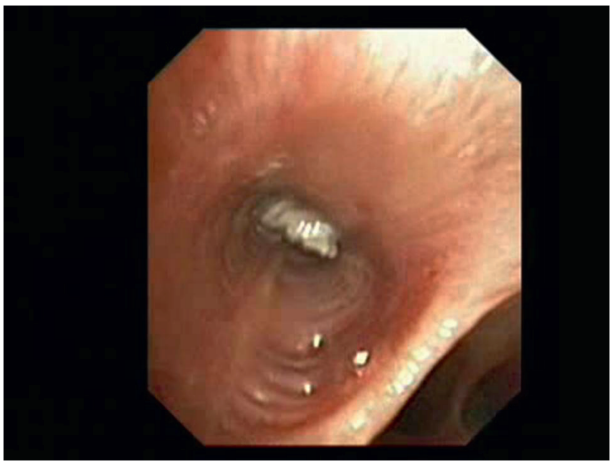

Fig. 4 – Shiny substance seen at repeat bronchoscopy.

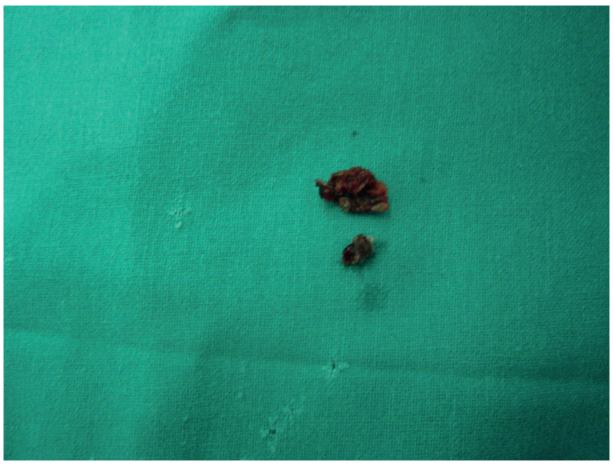

Fig. 5 – Blood mixed with glue.

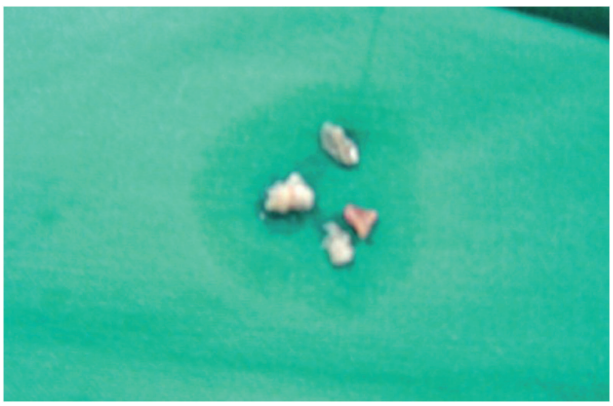

Fig. 6 – Expectorated glue.

4. Discussion

N-butyl-cyanoacrylate glue is a known drug used to seal defects/breaches in the bodily tissues and to occlude vessels in various medical specialties. It has been used in the management of recurrent retinal detachment caused by macular hole,[5] embolization of multiple pulmonary[6] and renal artery aneurysms,[7] in Behçet's syndrome, peripheral pulmonary artery pseudoaneurysm,[8] Intra-tumoral embolization of intracranial and extracranial tumors,[9] sclerotherapy in gastric ulcers[10,11] and rectal ulcers.[12] Endobronchial placement of glue has been effectively used for managing hemoptysis.[13,14] N-butyl cyanoacrylate is biocompatible glue used successfully for several haemostatic and sealing procedures. It has pro-thrombotic properties and leads to increased platelet aggregation & possible enhancement of local thromboxane production. When added to a physiologic medium such as blood or tissue, it polymerizes forming a hard substance.[15,16]

We instilled glue in 202 patients with mild to moderate and severe cases of hemoptysis with excellent results. There was 90.59% response, which is comparable to that reported earlier.[13] We had a very low failure rate because of accurate positioning of the catheter over the affected area which resulted in long term palliation. Shah et al[14] achieved success only in 50% of patients while the rest developed recurrence within 24 hours. In our study, recurrence of hemoptysis requiring a repeat procedure was observed in 19 patients (9.4%) out of which 14 (6.93%) recovered but 5 (2.47%) continued to have hemoptysis and required bronchial artery embolization.[17,18]

Our method of intra-bronchial application of glue is slightly different from other workers.[19] The glue is injected in totally dry conditions through the catheter which is passed through the bronchoscope because glue solidifies the moment it comes in contact with water, tissue or blood. We do not dislodge the clot and do not clean the scope with normal saline alone which may damage the equipment. Instead, we inject the glue into the orifice of the bleeding bronchial segment or into the clot without dislodging it so as not to ensue further bleeding. The clot fixed with the glue works as tamponade for the bleeding segment and helps in palliating hemoptysis. We clean the tip of the bronchoscope with acetone to dissolve any residual glue sticking to the instrument. There are times when glue will spill into the bronchoscopic channel which is a very risky situation and needs the bronchoscopic channel to be cleaned very gently with acetone. When removed from the working channel of the bronchoscope, the glue looks like a cast of the working channel (Fig. 7).

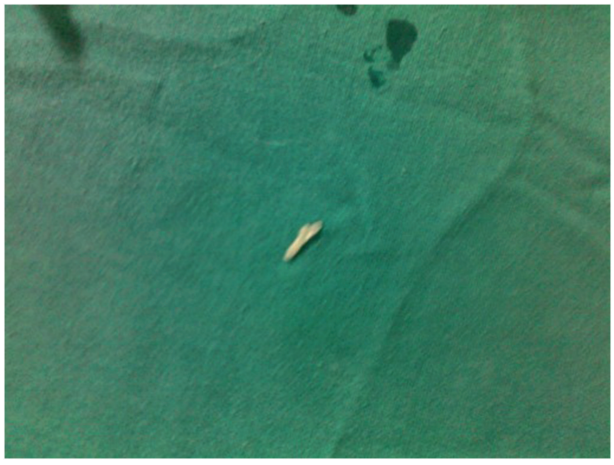

Fig. 7 – Cast removed from bronchoscope channel.

Success rate with glue is comparable to other forms of bronchoscopic interventional procedures. Hemoptysis was controlled in 94%–100% patients after endobronchial instillation of Fibrinogen-Thrombin.[20,21]

Conflict of interest

The authors have none to declare

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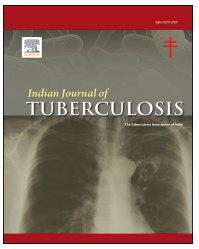

Original Article

Oxidative stress, antioxidant status and lipid profile in pulmonary tuberculosis patients before and after anti-tubercular therapy

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ABSTRACT

Background: Pulmonary tuberculosis (PTB) is a highly infectious dreadful disease caused by mycobacterium tuberculosis (MTB). Numerous studies reported free radicals activity, antioxidant status and lipid profile in PTB patients, but previous studies have lacunae in comparing the biochemical variables between before and after anti-tubercular therapy (ATT) supplementation to PTB patients. Hence, the present study was carried out to investigate oxidative stress markers, antioxidant status, lipid profile, liver function markers, and glycoprotein components in pulmonary tuberculosis patients (PTB) patients before and after 60 days of ATT.

Methods: This is a case-control study carried out with 100 healthy subjects and 110 PTB patients. All the patients diagnosed with sputum test and were positive for acid fast bacilli (AFB) were included for the study. An informed consent was obtained from all the patients.

Results: Our study found increased levels of oxidative stress markers, decreased enzymatic and non-enzymatic antioxidants, altered lipid profile in PTB patients as compared to healthy subjects before treatment and these levels were restored after clinical improvement with ATT. We also found increased concentrations of liver function parameters and components of glycoprotein in PTB patients. ATT refurbished lipid levels, antioxidant status and oxidative stress markers with decrease in liver function enzymes and glycoproteins in PTB patients.

Conclusion: Co-supplementation of antioxidants, along with ATT and inclusion of nutritious diet could be useful to reduce the pathogenesis of PTB and is warranted as a future study for the management of PTB.

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1. Introduction

Pulmonary tuberculosis (PTB) is a highly infectious dreadful disease caused by mycobacterium tuberculosis (MTB). MTB is a more causative agent than any other microbial agent causes more death globally and is influenced mainly by economic and nutritional status in developing countries.[1] It is reported that 8.6 million people developed TB and 1.3 million die of TB.[2] Further, the number of TB related deaths is expected to be increased from 3 million per year to 5 million by 2050.[3] Furthermore, its death rate is reported as over 95% in low- and middle-income countries.[4]

PTB is a multi-factorial disease and includes elevation of lipid peroxidation, depletion in antioxidants.[5,6] Mycobacteria induce reactive oxygen species (ROS) and reactive nitrogen species (RNS) production by activating both mononuclear and polymorph nuclear phagocytes by respiratory burst mechanism.[6,7] Oxidant-antioxidant status is necessary for the normal lung function. Imbalance in oxidant/antioxidant status can cause injury to host tissue and provokes an inflammatory state, contributing to immune suppression.[8] Evidence suggests that increased levels of free radical in PTB patients were found to have reduced immune response and antioxidant capacity contributing to the development of lung function abnormalities.[9–11] Studies report that lipid peroxidation by free radicals could reduce serum lipids.[9,12] Increasing evidence suggests that altered lipid profile and especially low cholesterol level is significantly associated with TB whereas high cholesterol bestows protection against patients infected with MTB.[12,13]

Glycoproteins are carbohydrate linked protein macromolecules found on the cell surface is essential for host-pathogen interaction and plays a crucial role in pathogenesis. Hexoses, hexosamine, sialic acid and glucosamine were the basic components of the glycosaminoglycan's and glycoprotein. These are involved in membrane transport, cell differentiation and recognition, the adhesion of macromolecules to the cell surface, secretion and absorption of macromolecules. Involvement of glycoproteins in disease progression by MTB is well documented.[14]

Liver is one of the vital organs plays a central role in metabolism of nutrients, drugs and detoxification of foreign substances. PTB is found to influence liver function enzymes such as AST, ALT and GGT during anti-tubercular therapy.[15,16]

Antitubercular therapy (ATT) is a kind of chemotherapy introduced and implemented to eradicate the dreadful killing disease. Drugs for TB could be first line or second line. These drugs have the greatest activity against TB. Numerous studies reported free radicals activity, antioxidant status and lipid profile in PTB patients but those studies have lacunae in comparing the biochemical variables before and after ATT supplementation to PTB patients. Hence, the present study was designed to investigate oxidative stress markers, antioxidant status, lipid profile, liver function enzymes and glycoprotein components before and after 60 days of ATT to PTB patients and they were compared with normal healthy subjects.

2. Materials and methods

2.1. Chemicals

Heparin, 2-thiobarbituric acid (TBA), Malondialdehyde bis(dimethyl acetal), trichloroacetic acid (TCA), 1,1',3,3'-tetraethoxypropane, 2,2'-dipyridyl, 2,4-dinitrophenyl hydrazine (DNPH), reduced glutathione (GSH), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), reduced nicotinamide adenine dinucleotide (NADH), phenazine methosulphate (PMS) and nitrobluetetrazolium (NBT) were purchased from Sigma Aldrich chemical company, St Louis, MO, USA. Kits for asparate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) were procured from Agappee diagnostics Pvt. Ltd., Kerala. Fine chemicals and reagents were acquired from Himedia Laboratories Pvt. Ltd., Mumbai, India or Sisco Research laboratories Pvt. Ltd., Mumbai, India.

2.2. Patients

This is a case-control study, which was carried out with 100 healthy subjects and 110 PTB patients of both sexes, aged between 18 and 55 years and free from HIV infection. The study was conducted in Rajah Muthiah Medical College and Hospital (RMMC&H), Annamalai University, Annamalainagar, India and approved by Institutional Human Ethics committee, RMMC&H. An informed consent was obtained from all the participants recruited the study.

All the patients diagnosed with sputum-test and were positive for acid fast bacilli (AFB) by direct microscopy based on Ziehl Neel-sen (ZN) staining were included for the study. Further, microbiological culture and radiological investigation (X-ray) were performed to confirm pulmonary infection. Clinical examinations were carried out for all the participants by a physician. They were free from other chronic illness and were normotensive.

Antituberculous treatment (ATT) was given to the PTB patients as per the guidelines of the Revised National Tuberculosis Control Program (RNTCP) and Direct Observed Short Course Chemotherapy (DOTS) strategy. Doses were adjusted as per body weight of patients. The first line drugs of an antitubercular drug regimen comprised of initial intensive phase therapy with four drugs isoniazid, rifampicin, pyrazinamide and ethambutol and/or streptomycin and were given to the PTB patients for 60 days.

2.3. Sample preparation

Blood samples were drawn from healthy subjects and PTB patients into heparinised tubes after an overnight fasting. Biochemical estimations were done in the erythrocyte and plasma. After plasma separation, the buffy coat was removed and the packed cells were washed thrice with physiological saline. A known volume of erythrocytes was lysed with 20mmol phosphate buffer (pH7.4).[17] Aliquots of erythrocytes were kept at 4 °C until used for further analysis.

2.4. Biochemical analysis

Oxidative stress markers such as thiobarbituric acid reactive substances (TBARS),[18] Conjugated dienes,[19] were measured. Activities of enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) were assayed in the hemolysate by standard procedures mentioned elsewhere.[20] Non-enzymatic anti-oxidants such as vitamins C and E and reduced glutathione (GSH) were quantified as described elsewhere.[21]

The plasma content of total cholesterol (TC) and triglycerides (TG) were measured by the standard methods described elsewhere.[22] High-density lipoprotein-cholesterol (HDL-C) was determined using a commercial kit while very low density lipoprotein-cholesterol (VLDL-C) and low-density lipoprotein-cholesterol (LDL-C) were obtained using Friedwald's formula.[23] Liver function biomarkers such as plasma protein,[24] total albumin,[25] were assayed. AST, ALT, ALP and GGT were analyzed using kits. Glycoproteins such as hexose, hexosamine, fucose and sialic acid were estimated in plasma by the standard procedures outlined elsewhere.[26]

3. Statistical analysis

Data was analyzed using SPSS statistical software (version 22, SPSS, Inc., Chicago, Illinois, USA). The results were expressed as means ± SD. Comparison was made using paired t-test. A value of $P < 0.05$ was considered statistically significant.

4. Results

Oxidative stress markers such as TBARS and CD were found to be increased in the plasma of PTB patients as compared to healthy subjects. ATT brought back these levels to near normal (Table 1). Depletion in the activities of enzymatic (SOD, CAT and GPx) and non-enzymatic antioxidants (GSH, vitamins C and E) were observed in PTB patients before treatment as compared to healthy subjects pointing out the imbalance in oxidant-antioxidant status. ATT restored these levels in PTB patients (Table 2).

Lipid profile in PTB patients was given in Table 3. PTB patients showed decrease in plasma content of TC and TG, and lipoproteins namely HDL-C, VLDL-C and LDL-C as compared to healthy subjects indicating altered lipid metabolism. ATT resulted in significant increases in these levels.

Table 4 shows the liver function parameters. The levels of TP, albumin and A/G ratio were decreased in PTB patients before treatment as compared to healthy subjects and after ATT, the levels were increased in PTB patients. PTB patients showed increased activities of AST, ALT, ALP, and GGT as compared to healthy subjects signifying liver damage. ATT significantly decreased these levels in PTB patients.

Table 5 represents levels of plasma glycoproteins in PTB patients. Significantly increased levels of protein bound hexoses, hexosamine, fucose and sialic acid levels were observed in PTB patients as compared to healthy subjects suggesting pathogenesis of MTB. ATT to PTB patients resulted in marked reduction of these levels.

Table 1 – Oxidative stress markers in PTB patients before and after treatment.

Parameters	Normal healthy subjects (n = 100)	Pulmonary tuberculosis Patients (n = 110)	
		Before ATT	After ATT
TBARS (nmol/ml)	3.55 ± 0.45	6.40 ± 0.68[a]	4.73 ± 0.49[a,b]
CD (μmol/ml)	0.82 ± 0.10	1.94 ± 0.20[a]	1.50 ± 0.16[a,b]

Values are means ± SD for each group.
[a] Significant as compared to healthy subjects ($p < 0.05$; P value based on paired t test).
[b] Significant as compared to before treatment ($p < 0.05$; P value based on paired t test).

5. Discussion

The present study assessed oxidative stress markers, enzymatic and non-enzymatic antioxidants, lipid profile, liver function markers and glycoproteins before and after ATT to PTB patients. The results of our study suggested higher oxidative stress markers and lower antioxidant capacity is the main culprit for pathogenesis of TB.

Oxidative stress (OS) results from an imbalance between the free reactive oxygen species (ROS) and the antioxidant mechanisms.[27,28] A higher risk of OS is reported in the lungs when compared to other organs.[29] Under normal circumstances, during the cellular metabolism due to production of ROS including hydrogen peroxide, hydroxyl radical, and superoxide radical, the lungs are exposed to the basal oxidative stress. In pulmonary TB, mycobacterium invades and replicates within the host macrophages. Then the infected macrophages undergo respiratory burst and produces high levels of ROS.[30] These enormously produced ROS are not adequately removed and these induces the Lipid Peroxidation (LP), rise in intracellular calcium ions and DNA damage.[31]

Several studies have found the link between OS and various lung disorders, including asthma, chronic obstructive pulmonary disease (COPD), acute pulmonary distress syndrome, and TB.[32,33] Further, poor dietary intake of micronutrients, malabsorption, poor immunity and altered metabolism might result increased free radical generation in PTB patients.[34] Combined effects of these cause oxidative stress which is also responsible for pathogenesis of tuberculosis.

Lipid peroxidation is a sign of tissue damage by free radicals and antioxidant enzymes are presumed to be an important endogenous defense against peroxidative destruction of cellular membranes.[35] Further, free radicals induced lipid peroxidation has gained more interest because of its involvement in several pathogenesis and could be assessed indirectly by measuring TBARS and CD.[35] In this study, elevated levels of TBARS and CD were observed in the circulation of PTB patients before treatment and could be attributed to either the progression of oxidative damage to the lungs or the deficiency of an antioxidant defense mechanism. These levels were lowered after ATT. Our findings are in agreement with previous studies.[35,36]

Table 2 – Activities of enzymatic and non-enzymatic antioxidants in PTB patients before and after treatment.

Parameters	Normal healthy subjects (n = 100)	Pulmonary tuberculosis Patients (n = 110)	
		Before ATT	After ATT
SOD (units[c]/mg of Hb)	3.19 ± 0.39	1.49 ± 0.17[a]	2.69 ± 0.27[a,b]
CAT(μmol of H_2O_2/min/mg of protein)	7.28 ± 0.85	4.11 ± 0.45[a]	5.81 ± 0.64[a,b]
GPx (μmolH_2O_2consumed/min/mg Hb)	7.69 ± 0.87	3.28 ± 0.35[a]	5.70 ± 0.59[a,b]
GSH(mg/dL)	34.88 ± 3.10	23.64 ± 2.05[a]	31.22 ± 2.68[a,b]
Vitamin C (mg/dL)	1.03 ± 0.12	0.52 ± 0.05[a]	0.88 ± 0.09[a,b]
Vitamin E (mg/dL)	1.31 ± 0.14	0.47 ± 0.05[a]	0.94 ± 0.10[a,b]

Values are means ± SD for each group.
[a] Significant as compared to healthy subjects ($p < 0.05$; P value based on paired *t* test).
[b] Significant as compared to before treatment ($p < 0.05$; P value based on paired *t* test).
[c] One unit of enzyme activity is the enzyme concentration which gave 50% inhibition of nitrobluetetrazolium reduction in 1 min.

Table 3 – Lipid profile in PTB patients before and after treatment.

Parameters	Normal healthy subjects (n = 100)	Pulmonary tuberculosis Patients (n = 110)	
		Before ATT	After ATT
TC (mg/dL)	164.62 ± 16.99	101.541 ± 17.43[a]	151.31 ± 13.06[a,b]
TG (mg/dL)	100.31 ± 10.99	71.03 ± 2.12[a]	89.33 ± 9.23[a,b]
HDL-C (mg/dL)	44.41 ± 4.28	24.07 ± 2.69[a]	35.03 ± 3.89[a,b]
LDL-C (mg/dL)	100.13 ± 17.94	63.27 ± 17.36[a]	98.41 ± 9.59
VLDL-C (mg/dL)	20.06 ± 2.19	14.20 ± 0.42[a]	17.86 ± 1.84[a,b]

Values are means ± SD for each group.
[a] Significant as compared to healthy subjects ($p < 0.05$; P value based on paired *t* test).
[b] Significant as compared to before treatment ($p < 0.05$; P value based on paired *t* test).

Table 4 – Liver function markers before and after treatment in PTB patients.

Parameters	Normal healthy subjects (n = 100)	Pulmonary tuberculosis Patients (n = 110)	
		Before ATT	After ATT
Total proteins (g/dL)	6.68 ± 0.68	5.50 ± 0.57[a]	5.81 ± 0.63[a,b]
Albumin (g/dL)	4.19 ± 0.38	2.56 ± 0.27[a]	3.28 ± 0.35[a,b]
Globulin (g/dL)	2.48 ± 0.39	2.93 ± 0.54[a]	2.53 ± 0.61[a,b]
A/G ratio	1.72 ± 0.31	0.91 ± 0.22[a]	1.39 ± 0.42[a,b]
AST (IU/L)	17.22 ± 2.14	44.75 ± 9.26[a]	27.64 ± 3.05[a,b]
ALT (IU/L)	16.36 ± 2.03	43.71 ± 7.38[a]	22.85 ± 3.75[a,b]
ALP (IU/L)	111.31 ± 13.77	142.42 ± 14.96[a]	113.40 ± 12.43[a,b]
GGT (U/L)	61.45 ± 8.09	90.53 ± 9.71[a]	73.21 ± 8.46[a,b]

Values are means ± SD for each group.
[a] Significant as compared to healthy and before treatment ($p < 0.05$; P value based on paired *t* test).
[b] Significant as compared to healthy and after treatment ($p < 0.05$; P value based on paired *t* test).

Table 5 – Levels of glycoprotein in PTB patients before and after treatment.

Parameters	Normal healthy subjects (n = 100)	Pulmonary tuberculosis Patients (n = 110)	
		Before ATT	After ATT
Hexoses (mg/dL)	103.44 ± 11.69	162.64 ± 16.56[a]	110.24 ± 15.79[a,b]
Fucose (mg/dL)	9.67 ± 0.92	22.38 ± 2.97[a]	14.84 ± 1.78[a,b]
Sialic acid (mg/dL)	65.84 ± 6.62	94.41 ± 1.07[a]	69.74 ± 1.21[a,b]
Hexosamines (mg/dL)	77.73 ± 8.24	110.55 ± 10.71[a]	98.65 ± 10.21[a,b]

Values are means ± SD for each group.
[a] Significant as compared to healthy subjects ($p < 0.05$; P value based on paired *t* test).
[b] Significant as compared to before treatment ($p < 0.05$; P value based on paired *t* test).

Antioxidative enzymes such as SOD, CAT and GPx are widely distributed in all cells and are present enormously in erythrocytes. These enzymes offer protection against O_2^- and H_2O_2 mediated lipid peroxidation thereby protect the host tissue from pathological events.[37] Multitudes of studies evidenced significant reduction in the activities of SOD, CAT and GPx in erythrocytes of PTB patients.[8–11] In support of this, we have observed similar kind of results in PTB patients before treatment.

Glutathione, vitamins C and E are integral components of the redox cycle.[38,39] Further water soluble (glutathione and Vitamin C) and fat soluble vitamin E might act combined to guard cells form oxidative stress induced damage.[40] Studies showed reduced concentrations of non-enzymatic antioxidants in patients with TB suggested increased lipid peroxidation is one of the prime contributing factors to the lower concentrations of antioxidants.[41,12,9] Additionally, malnutrition and exhaustion in an attempt to neutralise heavy capacity of free radicals are the other factors influencing antioxidant concentrations.[12] Our findings were similar with those of aforementioned literature. ATT refurbished the activities of antioxidants in patients with PTB and thereby protected lung tissue from oxidative damage.

PTB is associated with altered lipid metabolism. Decreased levels of lipid in the blood are considered to accelerate the development of lung diseases and are a major risk factor for infectious diseases. Numerous studies showed a strong correlation between decreased cholesterol and increased incidence of tuberculosis.[42,43] Reduced levels of lipid fractions in this study corroborate with Mosses et al, 2008; Reddy et al, 2009, Oyediji et al 2013; Casimir, et al,2013.[12,44,45,13] They suggested increased lipid peroxidation might be the cause for reduced lipid profile. This in turn could cause tissue and cell damages, consequently leading to wasting and weight loss in TB patients which may also be due to malabsorption and malnutrition.[44] Lower concentration of plasma lipids observed in the PTB patients in our study could be due to either diminished rate of lipid production or enhanced breakdown of lipids. These levels were increased significantly following ATT.

Albumin is a powerful scavenger of the phagocytic product like hypochlorous acid (HOCL) and provides the main plasma defense against this oxidant.[46] Further, it is an important component of plasma antioxidant activity that primarily binds to free fatty acids, divalent cations and hydrogen oxochloride (HOCL). Furthermore, as it is a negative acute phase protein its value in plasma may decreases during infection, injury or stress possibly as a result of the increased metabolic need for tissue repair and free radical neutralization.[47] Lower levels of total protein and albumin were observed in the present study, which is in accordance with other studies.[12,48] Further, they suggested anorexia, malnutrition and malabsorption were the other common findings in TB infection that may implicate with lower concentration of albumin. Increased concentrations of total protein and albumin were observed after the clinical improvement with ATT. In the present study, globulin level is significantly higher in PTB patients and decreased significantly following ATT. A decrease in the combination of protein and albumin increases the level of globulin in PTB patients which was similar to our findings.

The Liver enzymes ALT, AST, ALP and GGT are known to be elevated in a variety of clinical conditions associated with liver damage which indicates cellular damage and loss of functional integrity of cell membranes. We observed increased activities of AST, ALT, ALP and GGT in PTB patients before treatment as compared to healthy subjects. All these enzyme activities decreased significantly after ATT.

Clinical studies demonstrate that the concentrations of glycoprotein components are markedly increased in patients in a wide variety of pathological conditions like neoplastic diseases, clinical tuberculosis and cardiovascular diseases.[49] In this study, we have observed increased levels of plasma protein bound hexoses, hexosamine, fucose and sialic acid in PTB patients before treatment and this could be attributed to secretion or shedding of glycoprotein from the cell membrane into the circulation due to peroxidative damage of membrane proteins.[14] These levels were significantly reinstated after ATT.

6. Conclusion

The present findings showed oxidative stress, reduction in antioxidant status, lipid fractions whilst increase in liver function parameters and glycoprotein components in PTB patients before treatment as compared to healthy subjects. We are the first to report the changes in biochemical investigations before and after ATT. ATT restored lipid levels, antioxidant status and oxidative stress markers with decrease in liver function enzymes and glycoproteins in patients with PTB. There are few studies reported the effect of such nutritional supplementation on PTB. Treatment of PTB with special emphasis on nutritional supplementation might represent a novel approach for fast recovery in tuberculosis patients. In addition, raising nutritional status of population may prove to be an effective measure to control tuberculosis in developing/under developing regions of the world. Hence, in this study we suggest that co-supplementation of antioxidants, along with ATT and inclusion of nutritious diet/supplementation could be more beneficial and warrants further study for the management of PTB.

Conflicts of interest

All authors have none to declare.

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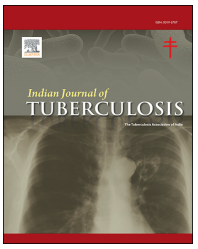

Original article

Tuberculosis (TB) intervention model targeting mobile population of truckers in Delhi, India

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ABSTRACT

Collaborative TB and HIV prevention and management activities are essential for reducing the burden of TB disease and achieving favorable outcomes by ensuring early initiation of antiretroviral therapy in the comorbid patients. The Mobile population of truckers and helpers is at higher risk of HIV and also TB infection.

Objective: The present study assessed the feasibility and opportunities for integrating TB screening and anti-tubercular drug dispensation services to truckers as an additional service utilizing the existing infrastructure and human resources of a targeted intervention (TI) based STI (Khushi) clinic and an integrated counseling and testing center (ICTC) operating at a transport hub and transshipment site in Delhi, India.

Methods: This exploratory operational research study was conducted at the Sanjay Gandhi Transport Nagar (SGTN), off the GT-Karnal highway, in North-west district of Delhi, the Indian capital city during May–Nov' 2016.

The proposed methodology for integration of comprehensive TB services within the existing STI/HIV services for the trucker population included a prevention and awareness component using interpersonal sessions, transporter meeting, one-on-one group session and IEC/BCC sessions utilizing a surround and engage technique. TB diagnostic testing and treatment services were provided through the collaboration with the TI/Khushi clinic and ICTC center staff aided by the field assistants.

Results: Overall, a total of 833 activity sessions were conducted during the study period among the truckers at the SGTN. During these sessions, 14644 truckers and 1444 other individuals were covered. A total of 297 truckers and 30 other people were referred for testing out of which 283 truckers and 33 others were tested for TB. Of these, ten truckers and four other individuals were found positive for TB.

Discussion: The present study provides the first patient (truckers) level evidence from India that routine, provider-initiated voluntary TB testing of truckers coming to avail services at STI and ICTC clinics for prevention and screening of HIV-AIDS is possible. The current

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practice of referral of HIV patients from the ICTC center to the chest clinic is inefficient since the opportunity costs and financial implications involved may deter patients from testing while the HIV negative but presumptive TB patients are likely to be missed. However, for this collaborative partnership to be successful, further investment regarding human and financial resources is necessary. Existing staff needs sensitization, training and proper incentives for conducting TB related IEC/BCC activities along with that for HIV-AIDS. Furthermore, the deployment of additional personnel is preferable for sputum collection and TB testing with the availability of early reporting at site.

1. Introduction

India has about 1.98 million new cases of tuberculosis (TB) annually, accounting for a fifth of new cases in the world – a higher number than in any other country with younger age groups being predominantly affected.[1,2] It is also estimated by the World Health Organization (WHO) that the risk of developing TB is 16–27 times higher in people living with HIV (PLHIV) than among those without HIV infection.[3] Among TB risk factors, HIV infection is considered the strongest.[4] Collaborative TB and HIV prevention and management activities are thereby essential for reducing the burden of TB disease and achieving favorable outcomes by ensuring early initiation of antiretroviral therapy in the comorbid patients. The Revised National Tuberculosis Program (RNTCP) - Phase III (2012–17) has the mandate for provision of universal access to quality services to all TB patients. Intensified TB-HIV collaborative services under RNTCP have been rolled countrywide since 2009 to combat TB amongst the PLHIV.[1] Nevertheless, there exists large sections of high risk, vulnerable and bridge groups lacking access to public health services for combating TB and HIV. Reaching these unreached populations to provide universal health access is pivotal for achieving the end-TB elimination targets.[5] The Mobile population of truckers is one such category which is at considerable risk of TB and the Human Immunodeficiency Virus (HIV) infection due to their high-risk behavior during absence from their home or native place which also renders the provision of services a challenge under programmatic conditions.[6,7] Nevertheless, this could be bridged by service delivery through non-governmental organizations (NGO) and civil society organizations.[8,9] However, in India sustainability of healthcare programmes for truck drivers is hindered because of knowledge gaps in trucker's needs and the impact of existing healthcare services on their health status. Moreover, to our knowledge, the feasibility of advancing TB services to the highly mobile and vulnerable trucker population within the ambit of existing national health programs is lacking.

The present study was thereby conducted to demonstrate a useful model of TB intervention in truckers and to formulate specific strategies for implementing TB services in this group. A preexisting nationwide HIV/AIDS prevention project 'Kawach' focusing upon the truck driving community in India provides services relating to behavior change communication, condom promotion and treatment of sexually transmitted infection (STI) through a network of 'Khushi' clinics.[10] The present study thereby assessed the feasibility and opportunities for integrating TB screening and anti-tubercular drug dispensation services to truckers as an additional service utilizing the existing infrastructure and human resources of a targeted intervention (TI) based STI (Khushi) clinic and an integrated counseling and testing center (ICTC) operating at a transport hub and transshipment site in Delhi, India.

2. Methods

Study Design

The present study was an exploratory type of operational research.

Study Area

This study was conducted at the Sanjay Gandhi Transport Nagar (SGTN), off the GT-Karnal highway, in North-west district of Delhi, the Indian capital city. The site is spread over an area of 77 acres that services as a vast transshipments, transport and workshop center housing a number of transport companies, hundreds of booking agents, body-building and repair workshops, and thousands of mechanics, welders, labor contractors, insurance agents, paint and spare part shops as well as dhabas (roadside eateries). On any given point, 2685–3000 trucks are parked at the SGTN. The truckers halting at SGTN consist of long distance (40%), short distance (35%) and local truckers (25%).

An integrated counseling and testing center (ICTC) for providing targeted intervention to the truckers is located in the SGTN, adjacent to which exists a Sexual Transmitted Infection (STI) clinic, branded as Khushi clinic which offers services to the truckers. The STI clinic is operated by the NGO Child Survival India and receives technical and financial support from the Delhi State AIDS Control Society. The STI clinic provides awareness, counseling, syndromic management and testing for HIV/AIDS. As the target population, i.e. truckers usually lack to medical facilities outside SGTN due to factors relating to distance, lack of time and issues pertaining to the safety of their trucks, the clinic services are highly acceptable to them. The clinic regularly serves an estimated 100000 population of truckers, helpers, and residents of SGTN.

The study activities were initiated and conducted during the period from May to November' 2016. Furthermore, several

focused group discussions were held at the STI clinic, SGTN among 80 truck drivers during a one month period before initiation of the study activities. A history of TB was reported by thirteen of them, of whom five had received treatment from local private practitioners while the remaining got treatment from their nearby DOTS center. The truck drivers opined that when diagnosed with TB they preferred to leave for their village after consulting the local physicians of the area as they perceived the need for adequate rest and nutrition was necessary for recovery from the disease. Furthermore, those TB patients who were initiated on DOTS failed to continue with it due to inconvenient timings, demands of the exacting regimen and all reported having eventually at some point of time have sought private TB care in spite of the costs involved.

This study explored the feasibility of provision of effective TB services for overcoming the barriers previously identified against continuum of care in the highly mobile and vulnerable trucker population by implementing it within the existing Targeted Intervention (TI) Centre/STI (Khushi) Clinic at the SGTN site which was already providing HIV-AIDS/STI-RTI services (Fig. 1). This collaborative project between the RNTCP, Delhi State AIDS Control Society, and the NGO Child Survival India thus offered TB services as an 'add-on' to the on-going HIV prevention, STI-RTI treatment strategies targeting the truckers and helpers.

The proposed methodology for integration of comprehensive TB services within the existing STI/HIV services was adopted as follows:

A. Prevention and Awareness Component: apart from the delivery of TB testing and treating services, the present study focused upon enhancing the awareness of TB disease pathology and symptoms, elimination of stigma and discrimination associated with TB, improvement of the TB treatment-seeking behavior and treatment adherence to DOTS therapy in the target groups.

Two field assistants of the project along with ten outreach workers of the Khushi (STI) clinic were trained for the Information Education Communication (IEC) and Behavioral Change Communication (BCC) activities for spreading awareness on TB among the target population. The material and tools required for conducting these IEC and BCC activities were pre-tested and developed specifically for the study and care was taken to ensure these were culturally appropriate and easily understandable irrespective of literacy status.

One on one and group sessions were conducted with the truckers and helpers that focused upon specific themes like awareness of TB symptoms and creating demand for TB services. The use of educational games like 'Snake & Ladder' and 'Savdhani Hatti, Durghatna Ghati' and health talks were very low-cost methods utilized in the present study for creating risk perception on TB amongst the truckers. Clients were also referred to the STI Clinic for accessing the HIV/TB services. Also, motivation and counseling sessions were held for the Medical doctor and Lab technician of the project along with the part-time Medical doctor at STI Clinic.

An innovative 'Surround and engage' technique was utilized to create an enabling environment for IEC and BCC activities. In the geographical enclosure, IEC material (wall paintings and posters) thematically explicitly designed for the

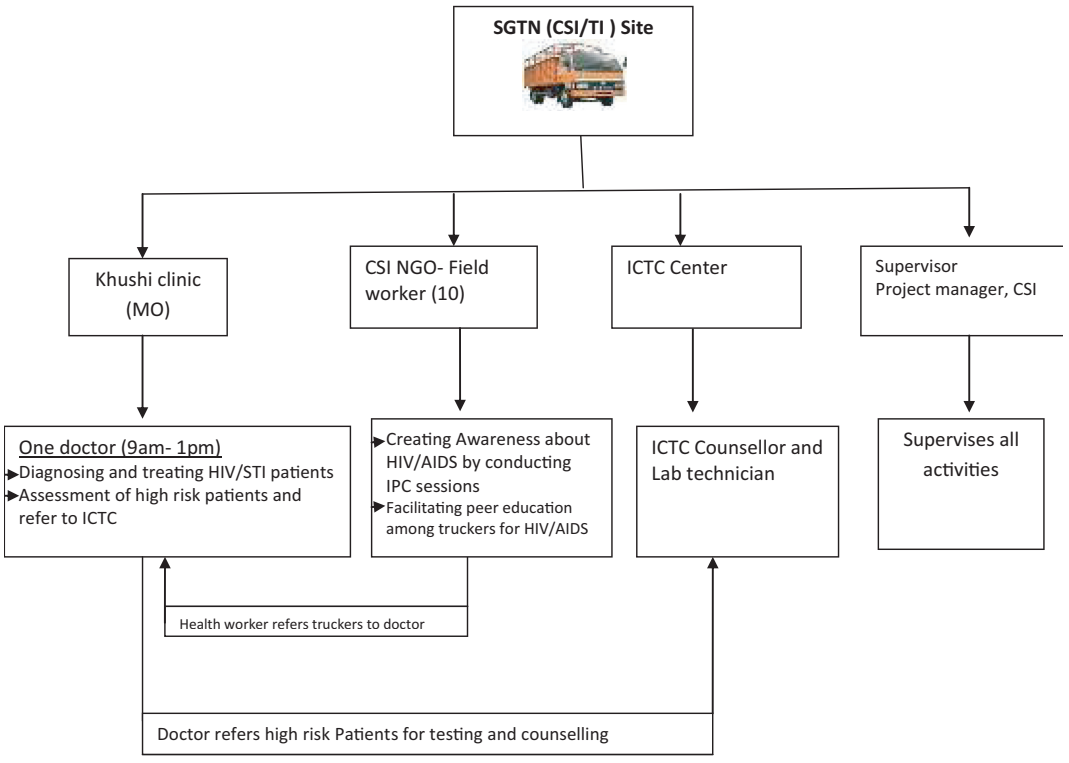

Fig. 1 – Working network of ongoing activities in STI/HIV control program of NGO (CSI) in SGTN.

target audience helped in securing their undivided attention towards the topics being explored at the time.

B. Diagnosis of TB at the STI Clinic, SGTN: The STI Clinic was suitably equipped for dispensing medicine and medical screening. The center was manned by trained staff including part-time medical doctor and paramedical workers. The counselors at the local area ICTC center were motivated to refer all PLHIV clients for TB screening to the medical doctor at the STI Clinic. Similarly, the clients referred by the field assistant suspected of having TB were referred to the Lab technician at STI clinic for sputum collection or accompanied by the field investigator to the Babu Jagjivan Ram Memorial (BJRM) chest clinic. For diagnosis, one good quality spot sputum sample was collected at the STI clinic by the lab technician who had been appropriately trained in sputum collection from the BJRM chest clinic. All the samples were transported to BJRM and subjected to Nucleic Acid Amplification Test (NAAT). The report of the sputum test was shared with the medical doctor on the same day. If the report was positive and Rifampicin sensitive, the project coordinator and field assistant tracked the client and accompanied him to the BJRM Clinic for categorization and treatment initiation. In case of the M.TB negative result, the project coordinator ensured client referral to BJRM Chest Clinic for further evaluation and final diagnosis of M.TB depending upon the results of the Chest-X Ray conducted at the same site. Similarly, for diagnosis of a presumptive extra-pulmonary TB case, the subject was referred and accompanied by the field investigator to the BJRM Chest Clinic.

Ethical approval for the study was obtained from the Institutional Ethics Committee, Maulana Azad Medical College & Associated Hospitals.

3. Results

To improve truckers understanding of tuberculosis, the present study conducted different activities like interpersonal communication sessions (IPC), street play, transporters meeting, ICTC and clinicians meeting (Table 1). These sessions were conducted to discuss basic principles of TB, measures for prevention and how it could be successfully treated and cured.

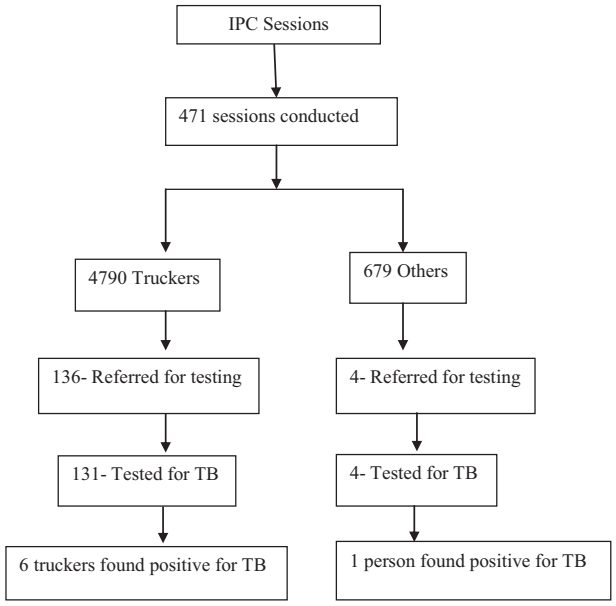

Fig. 2 – Outline of IPC sessions conducted in the study period.

The present study evaluates the feasibility for implementation of these IEC/BCC approaches in identification and of presumptive TB cases, prompt referral with treatment initiation as an add-on service through the existing programmatic staff and infrastructure from an STI clinic of the NACP operating in the same area.

1. Street plays: a total of seventeen street plays were conducted in the SGTN area of Delhi during May–Nov' 2016. These were brief, to the point and encoded TB related health awareness messages with the help of drama using catchy phrases and songs with a crisp script for making a lasting impression in the mind of the viewers. During the street plays, fifteen truckers and six other individuals were referred for testing of whom none were found positive for M.TB (Fig. 2).
2. Interpersonal (IPC) sessions: for promoting TB awareness were organized for the truckers through the medium of a video show using a projector. Each of the video shows were

Table 1 – Various activities conducted in the operational research project at SGTN.

S.No.	Activity	No. of activity sessions conducted	Total no of persons reached		No. of persons referred for testing		No. of persons tested for TB		No. of persons found positive	
			Truckers	Others	Truckers	Others	Truckers	Others	Truckers	Others
1.	IPC Session	471	4790	679	136	4	131	4	6	1
2.	Street Play	17	873	287	15	6	15	6	0	0
3.	Transporter's meeting	240	2619	321	70	9	68	9	3	2
4.	Clinic	105	5198	81	54	14	47	14	1	1
5.	ICTC	–	1164	76	22	0	22	0	0	0
	Total	833	14644	1444	297	33	283	33	10	4

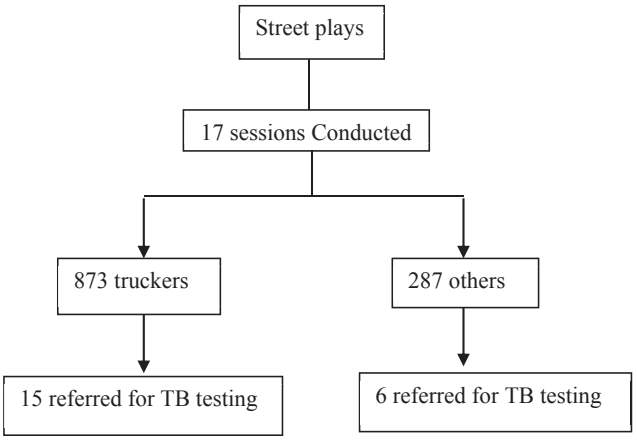

Fig. 3 – Outline of street plays conducted in the study period.

attended by 40–50 truckers and a total of 471 session were conducted. The content of the TB video show was medically accurate, culturally appropriate and readily understandable irrespective of the degree of literacy. The IPC sessions yielded a cumulative total of six diagnosed TB cases (Fig. 3).

3. Transporter meeting: in the present study, one to one and one to group meeting was conducted with transporters/brokers of SGTN, Delhi. These meetings were directed towards introduction of the study activities and services, motivation to send presumptive TB cases for testing. The issues discussed during the meetings were; scenario of TB in India and World, how to identifying TB patients, symptoms of TB, information about DOTS centers, etc. A total of 240 sessions were conducted during the study period at the end of which five individuals were referred and diagnosed with TB (Fig. 4).
4. STI (Khushi) Clinic and ICTC (Integrated counseling and testing center): STI (Khushi) clinic staff were trained in following-up M.TB diagnosed patients and dispensing medication to them on a regular basis. During the study period, a total of 105 IPC sessions were conducted in which 5198 truckers and 81 other individuals were covered and 54 truckers and 11 other individuals were referred for testing.

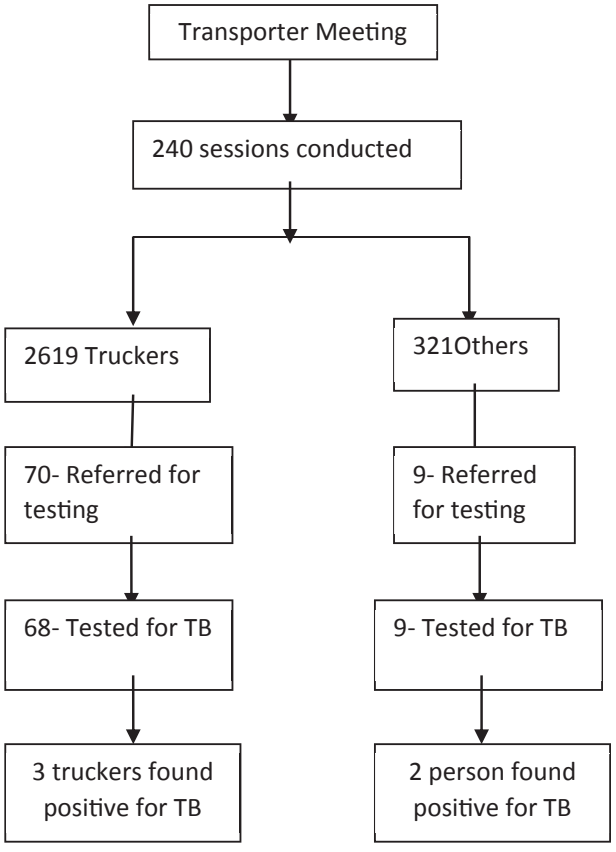

Fig. 4 – Outline of transporter meeting conducted in the study period.

At the ICTC center, the counselor referred the presumptive TB cases for TB testing. During the study period, a total of 1164 truckers and 76 other individuals were registered at the ICTC center, SGTN. Overall, 22 truckers were further screened for TB, and none were diagnosed with TB.

Overall, a total of 833 activity sessions were conducted during the study period among the truckers at the SGTN. During these sessions, 14644 truckers and 1444 other individuals were covered. A total of 297 truckers and 30 other people were referred for testing out of which 283 truckers and 33 others were tested for TB. Of these, ten truckers and four other individuals were found positive for TB.

4. Discussion

The present study provides the first patient (truckers) level evidence from India that routine, provider-initiated voluntary TB testing of truckers coming to avail services at STI and ICTC clinics for prevention and testing of HIV-AIDS is possible. Our TB service provider model utilized the existing target intervention center of the National Aids Control Organization (NACO) for providing TB awareness, diagnostic and treatment services with minimal interventions from the RNTCP. A variety of IEC/BCC activities like interpersonal communication sessions (IPC), street plays and transporters meeting using surround and engage techniques were successful in enhancing awareness of tuberculosis symptoms and reporting of presumptive cases for TB testing.

Several barriers and challenges against a successful joint TB-HIV intervention amongst truckers at the transport hub at SGTN were also identified in the present study. First, adequate human and financial resources are required for successful implementation of such interventions. In the present study, the staff at the STI (Khushi) clinic and the ICTC center were adequately motivated and sensitized. However, testing of sputum samples for M.TB and enrollment for treatment initiation were often delayed due to the resistance of junior staff of the chest clinic against the excess workload. Allocation of appropriate staff and proper incentives for the HIV program staff who are additionally engaged in the TB component is necessary for effective functioning of the venture. Second, there is a need for augmenting the infrastructure for rapid initiation of TB testing and generation of results from

the ICTC/STI clinic site. The current practice of referral of HIV patients from the ICTC center to the chest clinic is inefficient since the opportunity costs and financial implications involved may deter patients from testing while the HIV negative but presumptive TB patients are likely to be missed. Since the truckers are highly mobile, it is vital that their screening, testing, and test report availability should ideally happen in less time and preferably in a single visit. The truckers could not be expected to spend hours together in the waiting queues for OPD registration or X-Rays. The upgrading of ICTC/STI (Khushi) clinics at transport hubs like the SGTN with facilities of a full-time physician, Chest X-Ray, Cartridge Based Nucleic Acid Amplification Test (CBNAAT) machines is also highly recommended.

In the present study the field investigators, health providers and people involved in awareness program noticed higher interest and improvement in truckers adherence to TB therapy, which was attributed to the truckers (patients) having a better understanding of their diagnosis and rationale for taking their medicine. Moreover, after IPC sessions, many truckers developed a better understanding of why sputum collections were significant in both the disease diagnosis and subsequent monitoring of treatment effectiveness. Essential messages like "to seek treatment for cough of more than two weeks" and "free availability of TB diagnosis and treatment," etc., should be spread using different communication channels to increase awareness among truckers.

We believe that our pilot intervention result provides useful preliminary data and identifies the key issues in implementing an add-on TB testing model to the existing NACO/Kawach strategy for preventing and treating HIV-AIDS amongst truckers. Furthermore, evaluating the model developed in larger samples and for a longer duration will be required to demonstrate its efficacy in long-term improvement in awareness, diagnosis of presumptive cases, time to treatment initiation and treatment adherence in the trucker population.

In conclusion, our study highly recommends a partnership to provide TB services as an 'add-on' to the ongoing HIV program targeting the truckers and helpers. The existing infrastructure developed for targeted intervention centers can be used for TB diagnosis and treatment with the help of NGOs running the center. The current staff also need sensitization, training, and incentives for conducting TB related IEC/BCC activities along with that for HIV-AIDS. Furthermore, the deployment of additional personnel is preferable for sputum collection and TB testing with the availability of early reporting from the site itself. A fruitful partnership should be ensured between NGOs running the TI centers and the RNTCP.

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Revised National Tuberculosis Control Program, Government of Delhi.

Conflict of interest

The authors have none to declare.

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