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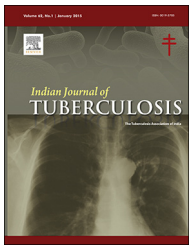

Editorial

Bedaquiline: Introducing a new drug to the MDR TB armamentarium

The approval of bedaquiline by the US Food and Drug Administration (FDA) in 2012 marked the first time that the US drug regulator had approved a novel antituberculous agent in over forty years, and the first time that any drug had been approved specifically for the treatment of multidrug-resistant tuberculosis (MDR TB). The issuance of the World Health Organization (WHO) interim guidance in 2013 for the use of bedaquiline under programmatic conditions presented bedaquiline national tuberculosis control programs (NTPs) an opportunity to re-assess their MDR TB treatment strategies and to address existing gaps especially around pharmacovigilance. The advocacy around the introduction of new drugs has also reminded governments to ensure that NTPs are adequately resourced to address the challenge of drug-resistant tuberculosis.

It is worth revisiting the path that bedaquiline has taken thus far. Following its discovery by Janssen scientists, and the characterization of antimycobacterial properties both in vitro and in animal studies, full clinical development of bedaquiline began about ten years ago with a series of phase 1 clinical trials that defined the pharmacokinetics and early safety parameters of the drug.[1,2] An early bactericidal activity (EBA) study showed that bedaquiline indeed had bactericidal activity albeit with a somewhat delayed onset.[3] This was followed by two randomized placebo controlled Phase 2 trials to demonstrate safety and efficacy. For the first of these trials (C208 Stage 1), which enrolled 47 subjects with newly diagnosed MDR TB, bedaquiline was added for 8 weeks to a standard WHO recommended MDR TB regimen, and for the second trial (C208 Stage 2), with a total of 160 newly diagnosed MDR TB patients, bedaquiline was added to the standard regimen for 24 weeks. Both studies achieved their efficacy endpoints, demonstrating that when added to a preferred MDR TB regimen, bedaquiline was superior to placebo in shortening time to sputum culture conversion (the primary endpoint) and increasing proportion of subjects achieved sputum culture conversion (secondary endpoint) at 8 weeks (for C208 Stage 1)[4] and at 24 weeks (for C208 Stage 2).[5] And at end of the pivotal C208 Stage 2 trial (at 120 weeks), nearly twice as many patients in the bedaquiline group as in the placebo group were cured (using the WHO definition of cure). The overall incidence of adverse events was similar between the bedaquiline and the placebo arms. There were 10 deaths (13%) in the bedaquiline group and 2 (2%) in the placebo group, with no causal pattern evident.[5] An additional study, whose main objective was to collect additional safety data, enrolled 233 subjects the majority of whom had previously received MDR TB treatment; 16% of these patients had XDR TB.[6] The mortality rate in this study was 6.9% (16/233) and other adverse events were consistent with those commonly associated with MDR TB treatment.

Based on data from the aforementioned trials, the US FDA granted bedaquiline 'accelerated approval' in December 2012 for the treatment of adults with multidrug-resistant pulmonary tuberculosis for whom an effective treatment regimen is not otherwise available. The FDA noted the complexity they faced in reviewing the bedaquiline data and explained their decision in an editorial published in the *New England Journal of Medicine*.[7] Bedaquiline has since been approved in over 40 countries including the 28 countries of the European Union, in Russia, in South Africa, and in India. The approval in India stipulates that bedaquiline be made available at a few selected sites identified by the Revised National TB Control Programme (RNTCP) and to a limited number of patients (up to 600 patients) on whom data will be collected to inform decisions on broader use; the RNTCP has started treating patients under this *conditional access program*. A phase 3 clinical trial, which is a post-approval requirement from some regulatory agencies, is currently under way. This trial (STREAM Stage 2) is being implemented in collaboration with the International Union against TB and Lung Disease, the study sponsor. The trial will explore the safety and efficacy of two bedaquiline containing regimens: an injection-free, 9-month regimen and a six-month regimen for MDR TB, which will also contain a second-line injectable drug.

Prior to its approval by regulatory agencies, Janssen initiated a pre-approval access program in 2011 to provide bedaquiline to patients with limited treatment options, i.e., patients with MDR TB that was also resistant to a fluoroquinolone or a second-line injection, or to both, i.e., extensively drug-resistant TB or XDR-TB. Over 800 patients from 47 countries received bedaquiline under this framework. In South Africa, the pre-approval access experience has served to inform programmatic rollout of bedaquiline post-approval.[8]

Bedaquiline interferes with mycobacterial energy production by inhibiting ATP synthase, a previously unknown mechanism of action among antimicrobial agents.[1] As such, one would not expect pre-existing resistance among prevailing *Mycobacterium tuberculosis* strains. But as the case with other antibiotics, however, variants with resistance mutations do exist that could be amplified through selection by inappropriate use of antimicrobial agents. In addition to target (ATP synthase) based resistance, antimicrobial resistance could also result from the effect of efflux pumps. Existence of these resistance-associated variants has been described for bedaquiline[9] although their clinical and public health implications are yet to be understood.

Guarding against the development of resistance to new drugs cannot be emphasized enough. The discovery and widespread use of streptomycin for the treatment of tuberculosis in the 1940s was quickly met by disappointment when development of resistance to the drug was documented. Combining streptomycin with other drugs mitigated but did not completely abrogate the acquisition of resistance during treatment. Recent approvals of new drugs such as bedaquiline for the treatment of MDR-TB should prompt us to reflect back to the dawn of anti-TB chemotherapy and remember the importance of using new drugs appropriately.

MDR TB continues to threaten the public health in many parts of the world. WHO states that only one in four MDR TB cases is detected and that only half of those who initiate treatment are cured. The long, arduous, and often toxic regimens used to treat MDR TB today are in part to blame for these reported poor outcomes.[10] The development of new anti-TB drugs has intensified the TB community's quest to simplify *and* shorten current MDR TB treatment regimens.

Recognizing that rapid and equitable access to new tools including new drugs will be crucial to meeting targets delineated in its new End TB Strategy, the WHO has advocated for rapid uptake of new drugs to optimize impact and has provided guidance on the implementation of MDR TB treatment regimens incorporating new drugs. In addition to specific guidance on the use of bedaquiline, WHO has published detailed policy advising countries to only introduce new drugs in the context of a functional national TB program organized according to WHO recommendations.[11] The WHO has set prerequisites that countries should have in place before introduction of a new TB drug or drug regimen(s). These prerequisites include laboratory capacity and case management modalities as well as the ability to clinically monitor patients undergoing treatment for MDR TB for adverse reactions.

Safe and appropriate use of these new anti-TB drugs will require that physicians treating MDR TB follow the relevant guidance provided in product package inserts and in the guidelines issued by public health authorities such as WHO and by local national TB programs. As more data become available, these guidelines should evolve to incorporate the new information and inform best practices.

We should celebrate the introduction of new drugs such as bedaquiline and we should all enlisted as responsible custodians of these new drugs for the benefit of today's as well as tomorrow's patients.

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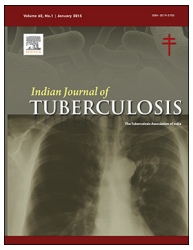

Original Article

CYP2E1 polymorphism, acetylator profiles and drug-induced liver injury incidence of Indonesian tuberculosis patients

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ABSTRACT

Objective: A polymorphism of *CYP2E1* may be directly associated with the development of INH hepatotoxicity. We conducted this study to evaluate the association between polymorphisms of *CYP2E1*, Isoniazid (INH) concentration and the acetylator status of INH in cases of Indonesian tuberculosis patients with drug-induced liver disease (DILI).

Methods: We conducted our study with a cohort design consisting of 55 Indonesian adult tuberculosis (TB) patients. Acetylating phenotypes were studied in using the metabolic ratio of plasma AcHZ/HZ. DILI was defined using CTCAV version 4.0. The allelic and genotypic frequency distributions of *CYP2E1* rs 3813867 were studied using the polymerase chain reaction – *amplification refractory mutation system* (ARMS) methodology.

Results: Patients with an INH concentration of more than 7 μg/mL showed a higher risk of developing DILI when compared with patients who showed a therapeutic range of 3–6 μg/mL INH (OR: 1.3, 95% CI: 0.2–8.2). Slow acetylators had a higher incidence of DILI when compared with rapid acetylators (OR: 4.6, 95% CI: 1.3–15.9). Meanwhile, subjects with GC had a higher risk of DILI incidence (OR: 4.3, 95% CI: 0.8–24.4).

Conclusion: Our study shows that polymorphisms of CYP2E1 and slow acetylator may have role in the DILI incidence.

1. Introduction

In 2015, the tuberculosis (TB) prevalence in the world reached 42% lower than in 1990. In 2014, of the 9.6 million of TB incidence, 58% were from South-East Asia and Western-Pacific region. Still, India, Indonesia and China had the largest number of cases from global total number (23%, 10% and 10%, respectively). The treatment success rate for newly diagnosed TB patients reached 86% in 2013 which was still sustained since 2015.[1] Treatment success is the key outcome of TB burden reduction. Some factors may influence the

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treatment success of TB, like hospital facilities, staffing education, population covered and centralized or decentralized health care system.[2]

The TB treatment regimen is a four-drug combination consisting of isoniazid (INH), rifampicin (R), pyrazinamide (Z), and ethambutol (E). This anti-tuberculosis therapy can cause adverse drug events. The most commonly reported adverse drug event of anti-tuberculosis drugs is hepatotoxicity.[3–5] Among these compounds, isoniazid (INH) strongly associated with anti-tuberculosis drug-induced hepatotoxicity (ATDH) or drug-induced liver injury (DILI). Previous reports showed INH induced liver injury or hepatotoxicity in approximately 5% of patients.[4–6] Other studies showed the development of ATDH in approximately 1 to 36% of patients.[7,8] INH is metabolized by *N-acetyltransferase-2* (NAT-2) into acetylisoniazid. Next, the metabolite is hydrolysed to *acetylhydrazine* (AcHZ) and *hydrazine* (HZ) by hepatic N-acetyltransferase-2 (NAT-2).[9] These compounds are then oxidized by cytochrome P450 2E1 (*CYP2E1*) to form intermediate hepatotoxins.[10,11]

There are large variations in the metabolism of the antituberculosis drug isoniazid, including polymorphisms of the *NAT-2* gene. This enzyme is markedly decreased in the livers of slow acetylators (SA). The elimination of INH follows a bimodal or trimodal distribution consisting of slow (SA), intermediate (IA) and rapid acetylators (RA). There is a strong correlation between these phenotypes and *NAT2* genotypes in Caucasians.[11–13] Previous studies have reported inconsistent results on whether slow or rapid acetylators are a risk factor for INH-induced hepatotoxicity.[9,13–15]

The objective of this study was to determine the association between *CYP2E1* polymorphisms and the development of DILI in Indonesians and determine the genotypes and phenotypes related to a change in the risk of DILI.

2. Materials and methods

2.1. Patients

This study used a cohort design. A total of 55 Indonesian adult patients with newly diagnosed TB at 20 Public Health Centres in the Provinces of Yogyakarta (10 Public Health Centres) and Lampung (10 Public Health Centres) were enrolled. The patient's recruitment was conducted from January until December 2013. The inclusion criteria included all adult patients (age >18 years) newly diagnosed as a pulmonary TB patient who were receiving category 1 TB medications and signed the informed consent for study participation. Exclusion criteria consisted of TB patients with HIV/AIDS, an AST and ALT two-fold higher than normal baseline concentrations, hepatitis or a history of hepatitis, a haemoglobin concentration <8 mg/dL, cessation of medication for more than 2 weeks, a history of kidney diseases, or refusal of blood sampling procedures and patients who refused to participate in this study.

All patients received a standard TB treatment for the first 2 months, including oral INH (300 mg), rifampicin (600 mg), pyrazinamide (20 mg/kg body weight), and ethambutol (800 mg). After 2 months of treatment, the patients were given INH and rifampicin for an additional 4 months. The total duration of antituberculosis treatment was 6 months. Serum alanine transaminase (ALT), aspartate transaminase (AST) and alkalyne phosphatase were measured before the antituberculosis therapy and then monthly until the end of treatment.

Genomic DNA was extracted from blood samples using a DNA Gene JET Genomic DNA Purification Kit (Thermo Scientific®, Nutrilab Pratama, Jakarta, Indonesia) according to the manufacturer's instructions. Analysis and Identification of SNP (−1055) C/G (Rs 3813867) was completed using an *amplification refractory mutation system* (ARMS) method. Two primer sets of *CYP2E1* (Rs 3813867) were used: forward I "5-GTA**C**AAAATTGCAACCTATG-3" to detect the *CYP2E1* polymorphic gene (F-primer – 1), forward II "5-GTA**G**AAAATTGCAACCTATG-3" to confirm the normal gene fragment and (F-primer – 2) reverse "5-ATCTTGTCTTTGTTGATCCC-3" (Genebank accession number P05181). The cycling conditions involved preliminary denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s, followed by a final elongation step at 72 °C for 2 min. The PCR products of single nucleotide polymorphism (SNP) (−1055) C > G (rs3813867) were obtained for 55 subjects. The results showed a band at 230 bp. After electrophoresis using a 2% agarose gel, samples were observed under UV light and imaged. Samples with homozygote CC (cytosine-cytosine) appeared using primer set Forward 1-Reverse (F1-R), samples with homozygote GG (guanine-guanine) appeared using primer set Forward 2-Reverse (F2-R), and samples with homozygote CG (cytosine-guanine) appeared using both primer sets F1-R and F2-R.

Venous blood samples were collected 2 h after drug administration. Determination of acetylator status was made using the AcHZ/HZ ratio with a *cut-off point* of 15.00. A slow acetylator (SA) was defined as an AcHZ/HZ ratio ≤ 15.00, and a rapid acetylator (RA) was defined as an AcHZ/HZ ratio >15.[16] The AST and ALT levels were measured with an automatic chemical analyser. Isoniazid and its metabolites, HZ and AcHZ, were measured using HPLC. Statistical significance was analyzed using correlation tests. DILI incidence was defined as an ALT and/or AST level above the upper limit normal value (ULN) listed in Common Toxicity Criteria for Adverse Events version 4.0 (CTCAE v. 4). The normal values of ALT and AST are 0–50 mg/dL and 0–33 mg/dL, respectively, for males. The normal values for ALT and AST are 0–34 mg/dL and 0–27 mg/dL, respectively, for females. Increased ALT and/or AST or an increased ALP were categorized as grade 1+, (>1.0–2.5 × ULN), grade 2+ (>2.5–5.0 × ULN), grade 3+ (>5.0–20 × ULN), or grade 4+ (>20.0 × ULN).[17]

This study was approved by the National Ethics Committee of the National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia, and written informed consent was obtained from all participants.

2.2. Statistical methods

Statistical analysis was conducted using the SPSS® statistical package, version 16.0. The odds ratio (OR) and confidence interval (CI) were calculated using binary logistic regression analysis. This analysis evaluated the risk and association of DILI, INH and acetylator status. Allele frequencies were

calculated, and the agreement between allele frequencies and Hardy–Weinberg equilibrium was tested using a chi-square test (d.f. = 1) for each locus.

3. Results

This study recruited 55 tuberculosis patients. Table 1 shows the demographics of the patients. There were 9 patients (16.36%) genotyped as CC (c1/c1), 29 patients (52.73%) genotyped as CG (c1/c2) and 17 patients (30.90%) genotyped as GG (c2/c2). The genotype frequencies were not significantly different from those predicted by the Hardy–Weinberg equation ($P > 0.05$). According to sex and age differences, the CG genotype was predominant over CC and GG. There were also no significant differences between ALT and AST means at baseline between males and females with different genotypes ($P > 0.05$).

Table 2 shows the association between INH concentration, acetylator status and DILI incidence. Among the 55 tuberculosis patients, there were 25 patients (45%) with DILI. There was no significant difference between INH serum concentrations and DILI incidence. However, patients with INH serum concentrations more than 7 μg/mL had a greater tendency to develop DILI when compared with subjects who had normal (3–6 μg/mL) and low (3 μg/mL) INH concentrations (OR: 1.3, 95% CI: 0.2–8.2). There was a significant association between acetylator status and DILI incidence. A total of 17 patients (30.9%) were identified as RAs, and the other 38 patients (69.09%) were SAs. SAs showed a higher risk of developing DILI when compared with RAs (OR: 4.6, 95% CI: 1.3–15.9).

Table 3 shows the distribution of INH serum concentration and acetylator status based on genotype. There were 10 patients (16.36%) genotyped as CC (c1/c1), 29 patients (52.73%) genotyped as CG (c1/c2) and 16 patients (30.90%) genotyped as GG (c2/c2). There were no significant differences in the frequencies of c1/c1, c1/c2, and c2/c2 genotypes between the patients with and without DILI. The heterozygous c1/c2 variant showed a higher DILI incidence when compared with the homozygous c2/c2 variant (OR: 4.3, 95% CI: 0.8–24.4 and OR: 2.5, 95% CI: 0.4–15.5, respectively). We did the stratified analysis according to the acetylator data to the INH serum concentration as the determinant; however, we did not find significant association ($P > 0.05$, data was not shown). Furthermore, the binary logistic regression analysis was performed; however we also did not find significant association ($P > 0.05$; data was not shown). After performing bivariate analysis, there was interesting pattern of results in this study, which are: patients who did not experience DILI had INH serum concentration between 0 and 7 ng/mL (OR; 1.8; 95% CI: 0.4–8.2). After stratified by genotyping, we found that the patients with GG genotype have more possibilities to experience DILI (OR: 0.7; 95% CI: 0.4–1.1). We conducted the stratified analysis according to acetylator status with DILI status as the determinant of INH serum concentration. We found that patients without DILI have lower serum concentration INH in slow acetylator (OR: 1.6; 95% CI: 0.3–7.6).

An INH serum concentration greater than 7 μg/mL was found to be predominant in allgenotypes. This pattern was also found for acetylator status. A SA status is dominant in all

Table 1 – Demographics of Indonesian tuberculosis patients according to genotype.

	Genotype			
	CC N (%)	CG N (%)	GG N (%)	
Sex				
Male	4 (44.5)	15 (53.6)	10 (55.5)	
Female	5 (55.5)	13 (46.4)	8 (44.5)	
Total	9 (100.0)	28 (100.0)	18 (100.0)	
Age (years)				
18–35	2 (22.2)	20 (71.4)	10 (55.6)	
36–55	5 (55.5)	3 (10.7)	6 (33.3)	
>55	3 (33.3)	5 (17.9)	2 (11.1)	
Total	9 (100.0)	28 (100.0)	18 (100.0)	
	Mean ± SD	Mean ± SD	Mean ± SD	P value
ALT (U/L) Baseline				
Male	21.8 ± 15.98	23.2 ± 16.81	20.8 ± 11.72	0.07
Female	16.4 ± 6.69	15.3 ± 12.15	14.6 ± 3.99	
AST (U/L) Baseline				
Male	19.5 ± 9.03	24.4 ± 13.54	23.4 ± 7.19	0.35
Female	24.8 ± 15.89	20.6 ± 9.78	16.2 ± 5.47	

Table 2 – Association between INH concentration, acetylator status and DILI incidence.

Variable		With DILI N = 25	Without DILI N = 30	Total N	OR (CI 95%)	P-value
INH concentration (μg/mL)	0–3	4	4	8	Reference	
	3–6	3	4	7	0.6 (0.03–8.9)	0.63
	>7	18	22	40	1.2 (0.2–8.2)	0.83
Acetylators status	Rapid acetylators	12	5	17	Reference	
	Slow acetylators	13	25	38	4.6 (1.3–15.9)	0.02*
Genotype CYP2E1	CC(c1/c1)	2	7	9	Reference	0.09
	CG(c1/c2)	16	13	29	4.3 (0.8–24.4)	
Genotype CYP2E1	CC(c1/c1)	2	7	9	Reference	0.09
	GG(c2/c2)	7	10	17	2.4 (0.5–15.5)	

OR: odds ratio; CI: confidence interval.
* Significant difference ($P < 0.05$).

Table 3 – Distribution of INH serum concentration and acetylator status based on genotype.

Variable		CYP2E1 genotype			
		c1/c1 N = 10 (%)	c1/c2 N = 29 (%)	c2/c2 N = 16 (%)	P-value
INH serum concentration (μg/mL)	0–3	1 (10.0)	4 (13.8)	3 (18.8)	0.70
	>3–6	3 (30.0)	2 (6.9)	2 (12.5)	0.53
	>7	6 (60.0)	23 (79.9)	11 (68.8)	0.83
Acetylator Status	SA	8 (80.0)	19 (65.5)	11 (68.8)	0.65
	RA	2 (20.0)	10 (34.5)	5 (31.3)	0.43

genotypes. The INH serum concentration had no significant influence on DILI risk.

4. Discussion

Our study shows that the CG genotype of *CYP2E1* (rs 3813867) is the most frequent in Indonesian tuberculosis patients. Moreover, according to the acetylator status, SAs had a higher incidence of hepatotoxicity when compared with RAs and SAs with the heterozygous c1/c2 variant (CG). Previous studies explained a significant association between the c1/c1 genotype and the risk of developing DILI by a higher *CYP2E1* activity in patients with the c1/c1 genotype and the inhibitory effect of INH.[18,19] Another study showed that the *CYP2E1**1A/*1A polymorphism may confer a greater risk of INH-induced hepatotoxicity in a heterogeneous ethnic population; however, the patients in this study were receiving monotherapy and potential interactions were not addressed.[19] A recent study from Chamoro et al. reported that the *CYP2E1* c1/c2 polymorphism did not show a significance.[20] Our study's findings are in line with previous studies which reported that acetylator status was a risk factor for DILI in tuberculosis patients.[9,14,15,21]

There have been several reports on the relationship between INH concentration and ATDH. A previous study showed an association between INH serum concentration and *NAT2* genotype in Chinese tuberculosis patients.[22] INH is hydrolysed by NAT-2 and oxidized by CYP2E1 to form a hepatotoxic substance.[23] However, our study confirmed that there is no association between these parameters. Our study reports that around 40% TB patients had higher INH serum concentrations, and 45% among them are experienced DILI. Our study is in line with previous data that showed SA status is significantly associated with DILI in the Indonesian population.[24] Our patients underwent a combination therapy for TB treatment, including INH, and drug–drug pharmacokinetic and pharmacodynamic interactions occurred. INH is known to have a biphasic effect on *CYP2E1* activity that consists of inhibition followed by induction. Many studies have reported on drug combinations that increase the incidence of antituberculosis drug-induced hepatotoxicity up to 35%.[25–28]

Huang et al. showed that *CYP2E1* by RsaI restriction polymorphism was associated with susceptibility to INH-induced hepatotoxicity.[20] INH is principally metabolized by N-acetyltransferase 2 (NAT-2) and cytochrome P450 2E1 (*CYP2E1*). NAT-2 plays a key role in the detoxification and elimination of drugs, and it is also involved in carcinogen metabolism.[29] Modifications in NAT-2 activity can result in the accumulation of precursors, such as HZ and AcHZ, leading to the development of hepatotoxicity.[30–32] *CYP2E1* is predominantly expressed in the liver where it activates (via oxidation) low molecular-weight lipophilic compounds, drugs, and procarcinogens,[33] and it is involved in INH metabolism and generates hepatotoxic intermediates.[34,35] The wild-type and mutated *CYP2E1* alleles have been named c1 and c2, respectively. There are several in vivo studies that have associated the c1/c1 genotype with increased transcriptional activity, protein levels, and enzymatic activity.[20,21] Therefore, patients with the *CYP2E1* (c1/c1) genotype generate more hepatotoxins. Other studies have found that the c1/c1 genotype may be a risk factor for INH-induced hepatotoxicity.[34,35] These data are in contrast with our present study that found c1/c2 may be a risk factor for INH-induced hepatotoxicity in SAs.

This study has some limitations as follows: we did not consider the complexity mechanism of antituberculosis-induced hepatotoxicity which was also interfered by roles of other genes such as *NAT2* and *GST*.[36] The other mechanism of antituberculosis-induced hepatotoxicity which we did not involve in this study was due to the rifampicin-INH interaction. Rifampicin may induce INH metabolism through CYP2E1 which also induces the hepatotoxicity.[37,38] The small sample size in our study is caused by the one criteria of the study is newly diagnosed TB patients, which only small number during the study period.

5. Conclusions

SAs were associated with an increased DILI incidence. A large-scale population study is needed to confirm the association between INH serum concentration, *CYP2E1* polymorphisms, acetylator status and DILI.

Conflict of interest

The authors have none to declare.

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Original Article

Rapid detection of extensively drug-resistant (XDR-TB) strains from multidrug-resistant tuberculosis (MDR-TB) cases isolated from smear-negative pulmonary samples in an Intermediate Reference Laboratory in India

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ABSTRACT

Background: Direct sputum smear microscopy is commonly used for diagnosing tuberculosis (TB). The objectives of the study were first, to determine the recovery of *Mycobacterium tuberculosis* in smear-negative sputum samples through liquid culture (using MGIT 960) and solid culture (using LJ slant) and second, to screen multidrug-resistant isolates through line probe assay and further third, to identify XDR isolates through MGIT second-line DST from these positive MDR cultures in Delhi region.

Methods: In this study, the sample size was 717 (sputum smear AFB negative and culture positive for *M. tuberculosis* complex by both solid and liquid culture methods) MDRTB suspects who were enrolled from January 2014 to December 2014 at the Intermediate Reference Laboratory in New Delhi Tuberculosis Centre, New Delhi. Rapid line probe assay was performed on all culture-positive samples, which were direct smear-negative specimens, and LPA-confirmed MDR samples were tested on MGIT 960 second-line DST for identification of XDR strains.

Results: An overall increase in the culture positivity (9.4%) among these smear-negative cases shows a good sign of recovery from *M. tuberculosis* infection in these samples. 717 (9.4%) positive cultures (MGIT + LJ) were subjected to line probe assay. Out of these 717 cultures, 9 (1.2%) were confirmed as NTM, 50 (7%) were MDR, 4 (0.6%) were mono-rifampicin resistant and 654 (91.2%) cultures were sensitive to both drugs Rif and Inh, respectively. Out of these 54 (50 MDR +4 mono-RIF resistant) cultures as screened by LPA, 1 (1.8%) was XDR, 10 (18.6%) were mono-ofloxacin resistant and 1 (1.8%) was mono-Kanamycin resistant. Sensitivity to both drugs KAN and OFX was seen in 42 (77.8%) cultures.

Conclusions: Since the bacterial load in direct smear-negative suspected MDR samples is less, it is important to recover mycobacteria by rapid liquid culture method in such samples. Initial screening for MDRTB is to be done in such cases by performing rapid molecular

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genotypic drug susceptibility test such as LPA. Baseline second-line DST is also done to rule out the XDR cases among them for rapid and better management of XDRTB patients.

1. Introduction

Mycobacteria, belonging to the family Mycobacteriaceae, present a great challenge in various regions of the world today. The emergence and spread of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) is a major medical and public problem threatening global health. MDR-TB is defined as TB that is resistant to the two first-line anti-TB drugs – rifampicin (RIF) and isoniazid (INH). XDR-TB strains are in addition resistant to fluoroquinolones and injectable second-line drugs. XDR-TB emerges through mismanagement of MDR-TB treatment.[1]

The multidrug-resistant TB (MDR-TB) crisis continues, with an estimated 4, 80,000 new cases in 2013. Worldwide, about 3.5% of all the people who developed TB in 2013 had this form of the disease, which is much harder to treat and has significantly poorer cure rates. Furthermore, extensively drug-resistant TB (XDR-TB), which is even more expensive and difficult to treat than MDR-TB, has now been reported in 100 countries. Seeing the heavy losses caused by the disease, rapid diagnosis of mycobacterial infections is critical; therefore, attempts to shorten the time needed for the detection of such organisms deserves attention.[2]

Conventional methods for mycobacteria culture and drug susceptibility testing are slow and elaborate, requiring sequential procedures for the diagnosis. During this time, patients may be treated inappropriately, drug-resistant strains may continue to spread, and amplification of resistance may occur. Therefore, rapid diagnosis and identification of MDR-TB or XDR-TB strains are prerequisites for the worldwide fight against TB.

The rapid transmission and emergence of tuberculosis and other mycobacterial diseases has made it mandatory for laboratories to quickly detect and identify mycobacteria from clinical samples. When conventional culture media are used, as many as several weeks of incubation and substantial technical labour were necessary for the recovery of organisms. Since it was first introduced, the BACTEC 460 TB system (Becton Dickinson Microbiology systems, Sparks, MD) has been the benchmark for rapid detection of *Mycobacterium tuberculosis* complex.[3] Today, however, a number of new fully automated instruments with less time for detection have been developed. The BACTEC MGIT 960 is a fully automated, non-radiometric instrument that incubates and simultaneously monitors 960 numbers of 7 ml MGIT culture tubes.

Therefore, in this study, an attempt was made to analyze the number of all drug-resistant cases among patients who are smear negative but still have viable mycobacterium, which is a probable cause of spread of secondary infection of drug-resistant TB in the community. Also, mycobacterium recovered from sputum smear-negative TB patients and the pattern of drug sensitivity/resistance observed in these cases can help in better management of drug-resistant TB patients.

The aim of the present study was to determine the recovery of *M. tuberculosis* in smear-negative sputum samples through liquid culture using MGIT 960 and to rapidly screen the drug resistance through line probe assay, and also, to conduct drug susceptibility testing for second-line drugs namely, kanamycin (KAN) and ofloxacin (OFX), in these isolates.

2. Materials and methods

2.1. Specimen collection and transportation

The present study was carried out from Jan 2014 to Jan 2015 in New Delhi Tuberculosis Centre, which is IRL (Intermediate Reference Laboratory) for Delhi state. Samples for testing were accepted from MDRTB suspects belonging to 15 chest clinics, which were linked to this laboratory. Collection of samples (5–10 ml) in sterile leak proof container is being carried out in the peripheral microscopic centres, and the samples were transported from the chest clinics to IRL. At IRL, AFB auramine staining and sputum smear microscopy were done initially to segregate smear-positive from smear-negative sputum samples.

2.2. Specimen processing

All sputum specimens were processed by N-acetyl L-cysteine–sodium hydroxide (NALC–NaOH) method (final NaOH concentration, 1%).[4] After decontamination, the concentrated sediment was re-suspended in 2 ml sterile phosphate buffer solution (pH 6.8) and smears were prepared and stained by the Auramine-O Fluorescent staining method.[5] 0.5 ml of all smear-negative decontaminated specimens was inoculated on to MGIT 7H9 broth medium for MGIT 960 liquid culture (Salman Siddique, 2007). These vials were incubated at 37 °C in MGIT 960 system.

2.3. Identification of M. tuberculosis complex (MTBC) strains from clinical specimens

For the identification and differentiation of MTBC strains, the Immuno-chromatographic Lateral Flow assay (SD Bioline TBAg MPT 64 Rapid) was performed on all positive MGIT tubes according to manufacturer's instructions with positive AFB results prior to DST for confirmation of *M. tuberculosis* complex.[6]

2.4. Genotypic DST

For LPA, 1000 μl from each MGIT-positive culture was centrifuged at 10,000 × *g* for 15 min, the supernatant was

discarded, and the pellet was re-suspended in 100 μl of lysis buffer, followed by incubation at 95 °C in a hot air oven for 5 min. Subsequently, 100 μl of neutralising buffer was added to each sample, which was followed by centrifugation at 13,000 × *g* for 5 min, and then the supernatant was transferred in a separate 1.5 ml eppendorf tube, and the pellet was discarded.

2.5. *Amplification assay*

Briefly, for amplification, 35 μl of Primer Nucleotide Mix (PNM provided with the kit), amplification buffer containing 2.5 mM $MgCl_2$, 1.25 U hot start Taq polymerase (Qiagen, Germany) and 5 μl of extracted DNA in a final volume of 50 μl were used. The amplification profile for direct patient material as described by the manufacturer was used for all sputum specimens. PCR was performed on ABI Thermocycler using the following protocol: First, the template DNA was denatured for 15 min at 95 °C, followed by 10 cycles consisting of 30 s at 95 °C and 2 min at 58 °C, with an additional 30 cycles consisting of 25 s at 95 °C, 40 s at 53 °C and 40 s at 70 °C. The final cycle consisted of an 8 min run at 70 °C.

2.6. *Hybridisation and detection*

Hybridisation was performed using the hybridisation kits, including reagents, a 12-well plastic tray and an instrument (Twincubator) as provided by the manufacturer.[7] After denaturation, the biotin-labelled amplicons were hybridised and an alkaline phosphatase-mediated staining reaction was observed in the bands where the amplicons and the probe had hybridised.[7]

2.7. *Interpretation of results*

The MTBDRplus (Hain Life Sciences, Germany) assay strip contains 27 reaction zones; 21 of them are probes for mutations and 6 are control probes for verification of the test procedures. For the detection of RIF resistance, the probes cover the rpoB gene, while for the INH resistance specific probes cover positions in katG and inhA.

The absence of at least one of the wild-type bands or the presence of bands indicating a mutation in each drug resistance-related gene implies that the sample tested is resistant to the respective antibiotic. When all the wild-type probes of a gene stain positive and there is no detectable mutation within the region examined, the sample tested is susceptible to the respective antibiotic. In order to give a valid result, all six expected control bands should appear correctly. Otherwise, the result is considered invalid.[7] After interpretation of the LPA results, MDR was defined as resistance to at least INH and RIF. Monoresistance is resistance to only 1 drug and polyresistance is resistance to two or three drugs excluding the INH–RIF combination.

2.8. *Phenotypic DST*

MGIT susceptibility test was performed according to the manufacturer's recommendations.[8,9] All 1- to 5-day-old liquid MGIT-positive *M. tuberculosis* cultures were used for DST. 0.5 ml of this suspension was used for testing of two second-line drugs [KAN (2.5 μg/ml), OFX (2.0 μg/ml)] and a 1:100 dilution for growth control was used. 0.8 ml of BACTEC 960 SIRE Supplement was added aseptically to each of the labelled MGIT DST tubes. 0.1 ml (100 μl) aseptically reconstituted KAN drug in the KAN labelled tube and similarly, added in the OFX drug tube.

3. Results

A total of 5960 samples from diagnosis (Dx) suspects and 5679 samples from MDR-TB follow-up (FU) were received in the year 2014. Of these 5960 Dx specimens, 3610 (60.5%) were smear positive and 2350 (39.4%) were smear negative (Table 1). Of 5679 follow-up (FU) specimens, 398 (7%) were smear positive and 5281 (93%) were smear negative (Table 1).

All the smear-negative diagnosis (Dx) specimens (2350) received from suspects of MDR-TB were put in MGIT. The mean time for detection (TTD) of *M. tuberculosis* complex was 14 days for BACTEC MGIT liquid culture medium. Contamination was found in 235 (10%) samples in MGIT even after single-time re-decontamination; 1410 (60%) samples turned out to be MGIT culture negative. LPA was not performed on culture-negative samples. 611 (26%) samples were found to be MGIT culture positive for MTB complex and 94 (4%) were negative for MPT64 antigen in Immuno-chromatographic Lateral Flow assay. These 94 samples were considered as non-tuberculous mycobacterium (NTM) (Table 2).

All the follow-up smear-negative cases (5281) were put in LJ (Lowenstein Jensen) medium for solid culture. The mean time for detection (TTD) of *M. tuberculosis* complex was 28 days for LJ medium. 264 (5%) samples were contaminated in LJ even after single-time re-decontamination. 4594 (87%) samples turned out to be LJ culture negative. LPA was not performed on these culture-negative samples. 106 (2%) samples were found to be LJ culture positive for MTB complex and 316 (6%) were negative for MPT 64 antigen in Immuno-chromatographic Lateral Flow assay. These 316 samples were considered as NTM (Table 3).

There was an overall increase of 9.4% in the culture positivity in both liquid and solid media, and among these,

Table 1 – Details of diagnosis and follow-up samples.

Samples	Smear (+)	Smear (–)	Culture	LPA
Dx = 5960	3610 (60.5%)	2350 (39.4%)	2350 (put in MGIT only)	611
FU = 5679	398 (7%)	5281 (93%)	5281 (put in LJ only)	106
Total=	4008	7631	7631	717

Table 2 – Rate of recovery of Mycobacteria using MGIT (liquid medium).

Samples in MGIT (*n* = 2350)	MGIT culture (+) = 611 (26%) MGIT culture (–) = 1410 (60%) MGIT culture contaminated = 235 (10%) MTBC (–) = 94 (4%)

Table 3 – Rate of recovery of Mycobacteria using LJ (solid medium).

Samples in LJ (n = 5281)	LJ culture (+) = 106 (2%) LJ culture (–) = 4594 (87%) LJ culture contaminated = 264 (5%) MTBC (–) = 316 (6%)

Table 4 – Detection of MDR in smear-negative and culture-positive (MGIT + LJ) samples.

Samples in LPA (n = 717)	Rifampicin sensitive = 647 (90.2%) MDR = 50 (7%) MTBC (–) = 9 (1.2%) Mono-RIF resistant = 4 (0.6%) Mono-INH resistant = 7 (1%)

Table 5 – XDR detection in MDR isolates detected by LPA in smear-negative and culture-positive samples by MGIT DST.

Samples in MGIT SLD (n = 54)	KAN + OFX sensitive = 42 (77.8%) XDR = 1 (1.8%) Mono-KAN resistant = 1 (1.8%) Mono-OFX resistant = 10 (18.6%)

smear-negative cases show a good sign of recovery of *M. tuberculosis* in these samples.

Out of these 717 cultures that were culture positive from both (MGIT + LJ) in line probe assay, 9 (1.2%) were confirmed as NTM (as negative for TUB band in line probe assay), 50 (7%) as MDR, 4 (0.6%) as mono-rifampicin resistant and 7 (1%) as mono-isoniazid resistant. 647 (90.2%) cultures were sensitive to both drugs RIF and INH, respectively (Table 4).

Among these MDR and mono-RIF resistant samples, 54 samples that were culture positive (MGIT + LJ) were put in MGIT second-line DST assay. Out of these 54 samples, 1 (1.8%) was XDR, 10 (18.6%) were mono-ofloxacin resistant and 1 (1.8%) was mono-kanamycin resistant. Sensitivity to both drugs KAN and OFX was 42 (77.8%) (Table 5).

4. Discussion

Tuberculosis can be diagnosed rapidly with the use of newer diagnostic techniques that are approved and recommended by WHO. Under the RNTCP programme, all the diagnostic techniques that are being used are adopted from WHO-endorsed techniques.

Although patients with sputum smear-negative TB are less infectious, they are more likely to die during or before diagnosis than patients with smear-positive TB, and contribute to TB transmission.[10,11]

Sputum microscopy continues to be the best tool for detection of infectious tuberculosis as it provides information about the extent of infection in the patient. It helps in categorisation of the patient for treatment and is an objective method to monitor the patient's progress. Moreover, new fluorometric and molecular diagnostic techniques have been developed and widely used in the World National TB control Programmes. It is estimated that 30–40% of all TB cases are diagnosed by these rapid methods.[12]

Liquid culture systems may also be up to 20% more sensitive than solid culture.[13] However, more rapid and more sensitive techniques have replaced traditional methods of direct examination and culturing for diagnosing mycobacterial infections. Among the most recent methods are isolator blood culture, hybridisation and amplification, and CBNAAT, each with its own advantages and disadvantages. Also, these are costlier compared to conventional culture technique[14] (WHO report, 2014).

In this study, an overall increase in the culture positivity (26%) among these smear-negative cases shows a good sign of recovery of *M. tuberculosis* in these patient samples. This study 's results correlate (26% MGIT culture positive) with a study of Mehndi hasan et al., in December 2013,[15] which shows 66.7% MGIT culture positive. Also, similar recovery rates were observed in some studies like Can Bicmen et al. (63.2% MGIT culture positive).[16]

It was recommended by Syre et al.[17] that a larger study with more smear-negative, culture-positive samples would be required to evaluate the LPA test performance with smear-negative samples. The present study has provided the previously absent data on the efficient use of the LPA test with smear-negative and culture-positive samples.

Occurrence of XDR among MDR cases, which were reported in culture-positive samples, proves that it is very important to diagnose XDR in smear-negative cases and culture-positive cases also. This data provides information on the percent of XDR cases that still need to be diagnosed, which is essential in National Drug Resistance Surveys.

TB culture and DST using only solid media can take 6 weeks or more due to the slow growth of the MTBC organism. Thus, the importance of accurately diagnosing MDR-TB or XDR-TB has been the driving force behind the identification of newer, more rapid diagnostics to detect drug resistance in TB.

Limitation of our study was that a small proportion of TB suspected patients were recognised since it was referral based and the patients included were those who came to our IRL. Thus, we might have missed patients who were out-patients/ not seriously ill/not opted to attend the hospital for care. The patient population selected for LPA testing for TB can vary according to the clinical findings, stage of the disease (i.e., whether they have received anti-TB treatment), the incidence of mycobacteria in that region, and the experience of the laboratory itself.

5. Conclusion

Early identification of drug resistance even in TB patients not previously treated will lead to better management of MDR and XDR TB patients.

Few important conclusions can be drawn from the present study. First, the culture recovery rate in smear-negative specimens was high and second, rapid screening of MDR by the use of LPA can reliably generate first-line drug susceptibility results. Further, DST for second-line drugs at the point of MDR TB diagnosis can help in better management of patients as early initiation of treatment can be made.

Conflicts of interest

The authors have none to declare.

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Original Article

Role of real-time PCR for detection of tuberculosis and drug resistance directly from clinical samples

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ABSTRACT

Background: Only a few studies done earlier in India reveal the utility of real-time PCR in detecting drug resistance in cases of pulmonary tuberculosis.
Objectives: The study was carried out to standardise real-time PCR (Quantitative real-time PCR, qPCR) targeting 16s RNA for the rapid detection of tuberculosis and its drug resistance from suspected TB patients.
Materials and methods: Sputum samples from 100 clinically suspected tuberculosis patients, after processing were subjected to microscopy, MGIT culture and qPCR. qPCR targeted 16sRNA for detecting *Mycobacterium tuberculosis* complex, *KatG* and *rpoB* genes for detection of resistance to isoniazid and rifampicin respectively. 1% proportionate method and Line probe assay (Hain Lifesciences, Nehren, Germany) were used to confirm the MDR isolates.
Results: The study showed positivity of microscopy, culture and qPCR for *M. tuberculosis* as 37%, 44% and 46% respectively. Sensitivity of 100% and specificity of 96.5% in the detection of *M. tuberculosis* was observed for qPCR in comparison to culture. MDRTB was detected in 14 cases whereas monoresistance to rifampicin and isoniazid was detected in 1 and 3 samples respectively.
Conclusion: Real-time PCR targeting *16sRNA*, *KatG* and *rpoB* is a sensitive, specific, rapid and reliable technique to detect pulmonary tuberculosis and its MDR status directly from the sputum samples.

1. Introduction

Tuberculosis remains a major public health problem because of its risk of transmission from infected person to a healthy one. India accounts for 23% of global burden and 15% mortality among an estimated 9.6 million tuberculosis cases all over the world.[1] Emergence of multi-drug resistance (MDR) and extensive drug resistance (XDR) strains is even more distressing. The conventional methods used for diagnosis are time-consuming

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and are of low sensitivity. Early diagnosis besides effective treatment of infected cases can help to control the transmission of this devastating disease.

The spontaneous acquisition of DNA sequence mutations is the primary genetic basis for the development of *Mycobacterium tuberculosis* drug resistance. Nearly 96% of rifampicin (RIF) resistance is due to the genetic alterations within the 81 bp rifampicin resistance determining region of the *rpoB* gene corresponding from codons 507 to 533 (*Escherichia coli* numbering system).[2] Nucleotide changes resulting in amino acid substitutions at codon 315 of *katG* (catalase-peroxidase) account for 60–70% of the clinical resistance to isoniazid (INH). Another less commonly seen mutation occurs in the promoter region of the *inhA* gene which accounts for up to 10–15% of the clinical resistance to isoniazid observed and are typically found in combination with additional mutations in *katG*.[3] Early detection of the drug resistance in *M. tuberculosis* from clinical samples is of utmost importance in the management of anti-tuberculosis therapy.

Many nucleic acid amplification techniques have been developed in the recent past, which have enabled the early detection of *M. tuberculosis* complex directly from the clinical samples along-with detection of drug resistance. Standard amplification techniques display high specificity but low and variable sensitivity. Quantitative real-time PCR (qPCR) was recently proposed for the detection of mycobacterial infection. It is a simple and fast method to detect the presence of *M. tuberculosis* in clinical samples[4] and can also help in detection of drug resistance using specific probes. This method has the advantage over simple PCR such as speed, robustness, reproducibility and a low risk of contamination. Most of the studies done earlier were carried out on pure isolates of *M. tuberculosis* grown in culture.[5] Scarcity of data from India has prompted us to evaluate the use of qPCR in early diagnosis of presence of *M. tuberculosis* and its resistance towards RIF, INH (commonly used drugs for treatment of tuberculosis) directly from clinical samples.

2. Materials and methods

This was a prospective hospital based study conducted in the tertiary care hospital of south-coastal Karnataka, India for a period of 2 years from September 2012 to August 2014. The study involved two consecutive days early morning sputum samples from 100 suspected cases of pulmonary tuberculosis (history of cough >2 weeks) attending the pulmonary medicine department in our tertiary care centre. The institutional ethical clearance was obtained for the study.

2.1. Sample processing for smear, culture and DNA extraction

Samples were transported to the microbiology laboratory and were processed using modified Petroff's method for decontamination.[6] All the processed samples were divided into three parts: one part was used for microscopy, second part was used for culture and DNA extraction was done from third part to be used later for qPCR. The smears were stained with auramine O and read by fluorescence microscopy. Culture was done in MGIT automated culture system (BD) for the presence of AFB and positive cultures were identified by presence of MPB64 antigen. A part of the sediment was stored frozen at −70 °C after decontamination till the DNA extraction procedure. The frozen sediment was later thawed, washed with Tris buffer and sterile distilled water to remove any traces of sodium hydroxide. Extraction of DNA was carried out using QiaAmp DNA mini kit [Qiagen, Netherlands] as per the user guidelines with increasing the incubation time to 2 h from 10 min at 56 °C. The DNA OD was checked after the elution using Qubit 9® 2.0 Fluorometer (Applied Biosystems – Invitrogen).

2.2. Real time PCR amplification

A set of primers and MGB probes were designed with the help of NCBI BLAST [shown in Table 1]. The assay consisted of 12.5 μl Taqman universal mastermix (2x), 1.25 μl of reconstituted primer-probe [18 μl FP (10 pmol) + 18 μl RP (10 pmol) + 5 μl Probe (6000 nmol) + 59 μl low TE buffer)], 7.25 μl nuclease free water and 4 μl of DNA per 25 μl reaction volume in separate tubes. The PCR conditions were 1 cycle of 95 °C for 10 min, 40 cycles of 95 °C for 15 s, 60 °C for 15 s and extension at 72 °C for 1 min and it was done on Applied Biosystems® 7500 Real-Time PCR System. All the assay reagents were obtained from Applied Biosystems – Invitrogen. All the 4 MGB probes were labelled with FAM and designed to hybridise and fluoresce only with the wild type DNA. Absence of fluorescence was associated with the presence of point mutations at the target gene. Drug susceptibility testing results of real time PCR in clinical isolates were also confirmed phenotypically by 1% proportionate method on LJ media and also by a line probe assay (Hain Lifesciences, Nehren, Germany).

Some of the samples showed threshold cycle (C_t) for *rpoB* gene more than 40 cycles. These samples were pre-amplified using nested PCR and later the product was diluted to original concentration of the sample DNA and run in real time PCR. The primers used for the pre-amplification of *rpoB* gene were [F] 5′ – GGG AGC GGA TGA CCA CCC A – 3′ and [R] 5′ – TGT AGT CCA CCT CAG ACG AG – 3′.

Table 1 – The set of primer and MGB probe sequences used in the study.

Name of the gene	Sequence
ITS sequence of 16s RNA	[F] 5′ – TGC GGC TGG ATC ACC TCC TT – 3′
Probe	[R] 5′ – CCG GCT CTC GCC CAC TAC AG – 3′
	NED – CTA AGG AGC ACC ACG AAA ACG CCC CAA CTG – MGB
katG(315) primers	[F] 5′ – TGG GCT GGA AGA AGC TCG TAT – 3′
Probe	[R] 5′ – GGA AAC TGT TGT CCC ATT TCG – 3′
	6-FAM – CAC CAG GGG CAT C – MGB
rpoB primers	5′ – GAA CAA CCC GCT GTC GGG GT – 3′
	5′ – GTG CAC GTC GCG GAC CTC CA – 3′
Probes:	6-FAM – TGA CCC ACA AGC GC – MGB
rpoB (524/529)	6-FAM – CAG CGC CGA CAG T – MGB
rpoB (529/533)	

2.3. Standardisation of the real time PCR

Specificity of qPCR assay with the ITS primer-probe sequence for the detection of *M. tuberculosis* was checked by using bacterial DNA of culture isolates such as *Mycobacterium fortuitum*, *M. kansasi and M. ulcerans* which did not show any amplification. Specificity of qPCR for drug resistance was checked by using monoresistant and MDR strains obtained from National Institute for Research in Tuberculosis, Chennai, India. Standardisation was done using serially diluted standard *M. tuberculosis* H37Rv DNA (1 ng, 0.1 ng, 0.01 ng, 0.001 ng, and 0.0001 ng) obtained from same reference centre and the detection limit of up to 0.0001 ng or 100 fg of the DNA for the ITS gene was observed with the MGB probes.

3. Results

A total of 37 out of the 100 sputum samples were smear positive by fluorescent microscopy and 44 samples were culture positive. Of the 44 isolates 10 were MDR, 3 were mono-resistant to isoniazid and 1 was mono-resistant to rifampicin phenotypically by 1% proportionate method and were confirmed by a line probe assay.

Real time PCR showed 46 samples positive for MTB with a sensitivity of 100% and a specificity of 96.5% in the detection of *M. tuberculosis* in comparison to culture.

Out of the 46 positive samples analysed by the real time PCR, 14 showed point mutations in the genes responsible for the drug resistance to one or more drugs [Table 2]. The number of strains detected MDR and mono-resistant were comparable to the conventional method. About 20 out of the 46 samples had to be pre-amplified with a site specific set of primer for *rpoB* gene. No mutations were detected in rest of the 32 samples.

4. Discussion

In case of *M. tuberculosis* the genetic basis for drug resistance is accumulative mutations.[2] Delay in the diagnosis of drug resistant tuberculosis by conventional culture and drug susceptibility testing has forced the use of molecular techniques which are able to detect the mutations in genes responsible for the drug resistance. The ability of molecular techniques to detect drug resistance directly from the clinical samples can enable the physician to decide about the patient management in time.

A sensitivity of 100% and specificity of 96.5% was observed in the present study for the detection of *M. tuberculosis* with varying CT values ranging from 25 to 35, which is inversely proportional to the initial concentration of DNA present in the sample. Real time PCR helped to detect additional 2 cases of tuberculosis as compared to culture. This may be due to low sensitivity of cultures as both the samples were negative by smear microscopy. Later clinical improvement was also observed in these two patients after antitubercular treatment. Swai et al.[7] observed a sensitivity and specificity of 38.1% and 74.5% for cultures in smear negative sputum samples. Rathore et al.[8] from Mumbai, with the use of hydrolysis probes observed a sensitivity of 100% for the detection of *M. tuberculosis* using IS 6110 specific primer and another study in Brazil by Lira et al.[9] showed 79.7% sensitivity and 94.8% specificity with the use of *IS 6110* Taqman assay. Kaur et al.[10] found a sensitivity of 87.2% and specificity of 98.3% for the real time PCR in the detection of MTB directly from sputum samples. A sensitivity of 92% for the detection of *M. tuberculosis* was observed by Seagar et al.[11] from Scottish mycobacteria reference laboratory. Helb et al.[12] in Vietnam observed 100% sensitivity and 84.6% specificity of solid culture in comparison to 71.7% specificity of liquid culture for a real time based

Table 2 – The results of 14 samples with drug resistance in culture and real time PCR.

S.no.	Culture for MTB	DST proportionate method	Ct values of RT PCR assays			
			ITS	*katG*315	*rpoB*526	*rpoB*531
1*	Positive	Sensitive	18.63	18.99	21.06	20.69
2*	Positive	MDR	24.15	ND	ND	31.57
3*	Positive	INH resistant	23.24	ND	30.53	32.25
4	Positive	MDR	33.93	ND	35.48	ND
5	positive	MDR	33.21	ND	34.91	ND
6	Positive	MDR	33.05	ND	36.0	ND
7	Positive	MDR	35.67	ND	35.87	ND
8	Positive	MDR	36.90	ND	36.41	ND
9	Positive	MDR	31.45	ND	33.70	ND
10	Positive	MDR	32.52	ND	34.13	ND
11	Positive	MDR	31.71	ND	ND	33.8
12	Positive	MDR	31.89	ND	33.42	ND
13	Positive	MDR	35.2	ND	38.87	ND
14	Positive	RMP resistant	34	35.1	ND	34.7
15	Positive	INH resistant	33.9	ND	24.96	27.34
16	Positive	INH resistant	34.2	ND	33.63	32.1
17	Positive	INH resistant	31.7	ND	29.94	27.94

1* 2* 3* are direct bacterial DNA isolated from culture of *M. tuberculosis* H37Rv, known MDR and isoniazid resistant isolate. 4–17 are samples with varying smear positivity.
ND – not determined/amplified (implies presence of resistance as the probes were designed to hybridise and fluoresce only with wild type).

Cepheid gene expert. Lemaitre et al.[13] in a comparative study with Amplified *M. tuberculosis* direct test (AMDT) found 90% sensitivity and 100% specificity for real time PCR in the detection of pulmonary tuberculosis.

Many drug resistance mutation detection studies are available for MTB using real time PCR based assays with differently labelled probes. We found a sensitivity and specificity of 100% in the detection of mutations in two regions [*rpoB* 526 (CAC-GAC) and *rpoB*531 (TCG-TTG)] of the 8 different cluster of mutations seen in the hotspot region of 81 base pair sequence for rifampicin resistance and *katG*315 (AGC-ACC) mutation which commonly occur in the drug resistant strains of this region. The results were confirmed by line probe assay.

MGB probes previously used by Doorn et al. for the detection of isoniazid resistance, showed inconsistent results with that of sequence data.[3] Ong et al.[14] from Singapore reported the sensitivity of 100% for the detection of rpoB mutations in the hot spot regions of the RRDR (rifampicin resistance determining region) gene and overall sensitivity of 89.3% for all the mutations occurring in and outside the RRDR region. He also observed a sensitivity of 98.1% and specificity of 83.3% for the detection of isoniazid resistance. Luo et al.[15] found 100% sensitivity and specificity for the detection of common mutations at the *katG*315 and *rpoB* genes by using dual labelled probes. Rathore et al.[8] reported that the results of their real time assay was 100% sensitive for the detection of resistance to isoniazid, but discordance in the rifampicin resistance as 2 out of the 40 samples showed mutations in the 81 base pair region which were pan-sensitive on phenotypic drug susceptibility testing. Paitek et al.[16] found a sensitivity and specificity of 98% and 100% for rifampicin resistance, 85% and 100% for isoniazid with the use of molecular beacons for rapid drug susceptibility testing. Ramirez et al.[17] found an overall sensitivity of 85% and a specificity of 98% for the detection of MDR TB strains by real time PCR using high resolution melt curve analysis. Rindi et al.[18] studied a 100% concordance of real time PCR against nucleotide sequencing for the detection of isoniazid resistance.

One of the limitations observed in the present study was 20 out of 46 samples were not amplified in the initial real time amplification for *rpoB* gene, hence a nested PCR was used to pre-amplify the *rpoB* gene. Wada et al.[19] had previously mentioned that 11 out of the 27 samples analysed in their study had to be pre amplified with a site specific primers. This may be explained by the amount and quality of the DNA which are very critical in the real time detection of drug resistance. But using nested PCR for pre-amplification may lead to increase in cost of testing and turn-around time for reporting. Another limitation with the present study is that we checked only for *katG* gene mutation in MDR isolates as other less known mutations in *inhA* and *ahpC* genes are also known to cause isoniazid resistance in *M. tuberculosis*. Though many studies have highlighted the use of melt curve analysis for the identification of drug resistance mutations in *M. tuberculosis*, we found a higher sensitivity and specificity of MGB probes for the same.

To conclude the use of qPCR can help to detect cases of MTB that are smear negative because of its higher sensitivity and can provide early results. Use of qPCR for detection of drug resistance depends on the specificity of probes used for mutations leading to drug resistance.

Conflicts of interest

The authors have none to declare.

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Original Article

PCR targeting IS6110 in diagnosing tuberculosis in children in comparison to MGIT culture

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ABSTRACT

Background: Diagnosis of tuberculosis (TB) in children is difficult in children especially in extrapulmonary tuberculosis (EPTB). This study was conducted to evaluate the use of polymerase chain reaction (PCR) targeting IS6110 in the diagnosis of TB in children with pulmonary TB and EPTB and also to compare its performance with MGIT 960 culture and conventional microscopy.

Methods: A total of 142 cases (50 pulmonary, 92 extrapulmonary) of suspected TB patients <15 years of age were included in the study. The clinical specimens obtained from these cases were subjected to Ziehl–Neelsen staining (ZN), MGIT 960 TB culture and PCR targeting insertion sequence IS6110. Sensitivity and specificity of PCR were calculated in pulmonary and extrapulmonary specimens. The results were compared to MGIT culture.

Results: PCR targeting IS6110 sequence had sensitivity of 69.01% in various clinical specimens which was significantly more than MGIT culture showing a sensitivity of 47.41% ($p < 0.05$). Sensitivity of PCR IS6110 in extrapulmonary specimens was 65.21% which was lower than sensitivity in pulmonary specimens (76%) but was not statistically significant ($p > 0.05$).

Conclusions: Diagnostic efficacy of PCR IS6110 in pulmonary and extrapulmonary TB cases was similar. PCR using IS6110 primer had significantly better efficiency than MGIT culture in diagnosing TB in children.

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1. Introduction

Tuberculosis (TB) is among top 10 causes of death in children worldwide. According to WHO Global Tuberculosis Report 2013[1] an estimated 550,000 TB cases were reported among children under 15 years of age in India accounting for 26% of cases. Diagnosis of TB in children is often challenging due to vague clinical manifestations and paucibacillary nature, making conventional methods less sensitive for detecting mycobacteria tuberculosis.[2] Studies have shown sputum microscopy for acid-fast bacilli to be positive in less than 15% of cases and yields of cultures to be about 30–40%.[3] Serological tests are currently not recommended for diagnosis of TB[4] polymerase chain reaction (PCR) has been found to be more useful in diagnosing TB with sensitivity ranging from 70% to 90% as reported in different studies.[5]

Several *Mycobacterium tuberculosis* specific DNA sequences have been evaluated in different laboratories including MBP-64, 65 kDa antigen and IS6110.[6] The repetitive nature of IS6110 insertion sequence in *M. tuberculosis* genome makes it an attractive target for PCR amplification.[7,8] Several studies have been done to evaluate the efficacy of IS6110 sequence for the diagnosis of TB.[6,9,10] Confirming the diagnosis of TB in children especially extrapulmonary TB is more difficult. In this study, diagnostic efficacy of IS6110 based PCR assay was evaluated in clinical specimens obtained from pulmonary and extrapulmonary TB subjects. Also efficacy of PCR IS6110 was compared with MGIT 960 TB culture method in various clinical specimens of TB cases.

2. Methods

This prospective observational study was conducted in the department of Pediatrics, S.N. Medical College, Agra and National JALMA Institute for Leprosy and Other Mycobacterial Diseases (Indian Council of Medical Research), Agra from March 2013 to February 2015. Subjects <15 years attending the department with clinically suspected TB were included in the study. The study was approved by the ethical committee of the institute and written informed consent was taken from guardians of the study subjects.

Clinical and demographic details were recorded for all the study subjects (age, gender, history of contact, fever, cough, weight loss, other relevant history). Complete blood count, chest X-ray and tuberculin test were done in all subjects. CT scan head and USG abdomen were done where required for the diagnosis. Clinical specimens obtained from these cases (CSF, gastric lavage, sputum, pleural fluid, ascitic fluid and lymph node aspirate) were subjected to biochemical cytological tests and histopathological examination. Microscopy was done using Ziehl–Neelsen (ZN) staining and gram staining. MGIT 960 culture system and PCR targeting IS6110 were performed in all specimens. MGIT 960 culture method consisted of tubes filled with modified middlebrook 7H9 broth base to which clinical specimens were added and incubated at 37 °C. Mycobacteria consumed oxygen in the tube and caused fluorescent material to glow which is detected with the help of automated BACTEC MGIT 960 system. PCR was performed targeting IS6110 sequence.[6] Forward and reverse primers used were (5′-CCTGCGAGCGTAGGCGTCGG-3′) and (5′-CTCGTCCA-GCGCCGCTTCGG-3′).

The subjects were diagnosed as suspected TB cases using National Guidelines on Pediatric Tuberculosis[11] based on the clinical features and laboratory investigations. Patients having alternative diagnosis were taken as controls.

3. Statistical analysis

Sensitivity and specificity of PCR IS6110, MGIT 960 culture and ZN staining were obtained in various clinical specimens. Negative predictive value (NPV) and positive predictive value (PPV) of PCR IS6110 and MGIT 960 system were calculated. Degree of agreement between these two tests was calculated using Cohen's kappa method. Chi square test was applied to compare the results of PCR IS6110 in pulmonary and extrapulmonary specimens. Chi square test was also applied to compare the results of PCR IS6110 with MGIT 960 culture. Statistical analysis was done using software SPSS version 17. p value <0.05 was taken as significant.

4. Results

162 cases were evaluated during the study period; of which 142 cases were taken as TB suspects based on clinical features and laboratory reports and 20 cases with alternative diagnosis were taken as controls. Non-repetitive clinical specimens were obtained from the cases (50 from pulmonary specimens, 92 from extrapulmonary specimens). The clinical profile of the study subjects is given in Table 1. Age of the subjects ranged from 1 month to 15 years (mean = 4.74 years) with male:female ratio of 1.6:1. History of contact was present in 42.9% cases. Only 27.5% of cases had received BCG vaccination. Tuberculin test was positive in 33.75% of cases. Most of study subjects were undernourished (63.15% ≤ 5 years had weight for age < −2 SD and 59.52% > 5 year had BMI < −2 SD). Chest X-ray in pulmonary cases showed hilar adenopathy as the most common finding seen in 34 (68%) cases followed by pulmonary infiltrates in 25 (50%) cases, and miliary shadows in 11 (2.2%) cases and cavitation in 3 (0.6%) cases. USG finding obtained in abdominal TB cases showed ascites in all the 11 cases followed by enlarged lymph nodes (>10 mm in short axis) in 8 (72.7%) cases and mesenteric thickening (>15 mm) in 6 (54.5%) cases. CT scan findings in CNS TB cases showed hydrocephalus in 58 (90.6%) cases followed by basal exudates in 42 (65.6%) cases and infarcts in 28 (43.75%) cases.

Cytology and biochemical results of CSF obtained in 64 CNS TB cases showed cell count to range from 64 to 450/mm^3 with predominance of lymphocytes. Proteins were found to be >60 mg/dl in 58 (90.6%) cases. Ascitic fluid from abdominal TB (11 cases) and pleural fluid from tubercular pleural effusion (13 cases) showed cell count >100/mm^3 with predominance of lymphocytes in all the cases. In pleural fluid samples, proteins >3 g% was found in 11 (84.6%) cases. Ascitic fluid showed serum/ascitic albumin difference to be <1.1g% in 9 (81.8%) cases. Histopathology of the 4 lymph node aspirates showed granulomatous changes, of which 3 demonstrated caseation

Table 1 – Clinical profile of study subjects (n = 142).

Parameter		Number	Percentage
Age	4–≤8 years	56	39.4%
	8–≤12 years	23	16.2%
	>12 years	17	12%
Sex	Male	87	61.26%
Positive contact history	Positive	61	42.9%
History of previous ATT	Present	27	19%
Malnutrition			
<5 years	Wt for age <−2 SD	30	46.15%
>5 years	BMI < −2 SD	32	40.5%
BCG	Vaccinated	39	27.5%
Tuberculin test	Positive	48	33.8%
	>15 days	119	83.8%
Type of tuberculosis	Pulmonary TB	57	40.2%
	CNS TB	64	45.1%
	Abdominal TB	11	7.6%
	Others	10	7.1%

Table 2 – Comparison of MGIT culture, PCR IS6110 and Ziehl–Neelsen in various clinical specimens.

Sample type	MGIT culture positive *n* (%)	PCR positive (IS6110) *n* (%)	Ziehl–Neelsen microscopy *n* (%)
Sputum (*n* = 24)	15 (62.5%%)	18 (75%)	4 (16.6%)
G. aspirate (*n* = 26)	11 (42.3%)	20 (76.9%)	5 (19.2%)
CSF (*n* = 64)	26 (40.6%%)	41 (64.1%)	3 (4.6%)
Pleural fluid (*n* = 13)	6 (46.15%)	8 (61.5%)	0
Ascitic fluid (*n* = 11)	6 (54.5%)	9 (81.8%)	0
Ln-aspirate (*n* = 4)	2 (50%)	2 (50%)	2 (50%)
Total (*n* = 142)	66 (46.4%)	98 (69.01%)	14 (9.8%)

with central necrosis. Comparison of sensitivity of ZN smear, MGIT culture and PCR IS6110 in various clinical specimens of study cases is shown in Table 2. Sensitivity of PCR IS6110 (69.01%) was significantly higher than that of MGIT culture (46.47%) (chi square = 14.9, *p* value <0.05). Specificity of PCR reached 100% as none in the control group were positive for PCR. PPV and NPV of PCR IS6110 was 100% and 31.25%, respectively, whereas PPV and NPV values of MGIT were 100% and 20.83%, respectively. Overall concordance by Cohen's kappa method gave good agreement between PCR and MGIT methods (kappa value = 0.68). Sensitivity of PCR was higher in pulmonary specimens (76%) as compared to extrapulmonary specimens (65.2%); however there was no statistically significant difference (chi square = 1.96, *p* value >0.05). Sensitivity of MGIT culture and ZN microscopy in extrapulmonary specimens was 43.5% and 5.4%, respectively.

Of the 20 control samples, five had pyothorax and two had ascites due to chronic liver disease, 10 had viral encephalopathy and 3 had pyogenic meningitis. Microscopy results on gram staining demonstrated gram positive cocci in 3 cases of pyothorax and 3 cases of pyogenic meningitis; 2 cases of pyothorax showed gram negative bacilli. ZN staining, MGIT culture and PCR IS6110 showed negative results in all these cases.

5. Discussion

TB even today, remains a major health problem among children worldwide and in India. In our study extra pulmonary cases outnumbered pulmonary TB cases. Majority of them were tubercular meningitis (69.56%), probably as the cases were taken from tertiary referral hospital. Only 27.5% of patients had received BCG vaccine, which is also a probable explanation for more number of severe forms of TB cases registered in the study. Tuberculin test was positive in 33.75%, which was lower than 52.3% reported by Udani.[12] This could be probably due to co-existent malnutrition in study group.

In the present study, the PCR targeting IS6110 sequence showed sensitivity of 69.01%. Negi et al.[13] and Ogusuk and Salem[14] reported, respectively, 83% and 92.1% sensitivity of PCR targeting IS6110 in their studies in various clinical specimens.

Conventional methods have shown sensitivity of 20% in our study which is comparable to other studies.[2,3] Solid culture, though considered to be the gold standard, requires about 6–8 weeks time for positive reports, hence delaying initiation of therapy. Liquid culture such as MGIT 960 TB system provides for early detection of mycobacteria. Overall sensitivity of MGIT culture was 46.4% in this study which was comparable to previous studies (34.10% and 41%) respectively.[15,16] Our study has shown sensitivity of PCR IS6110 to be 69.01% which is higher than sensitivity of ZN smear microscopy and MGIT culture in detecting mycobacteria as also seen in another study showing sensitivity of ZN microscopy, MGIT culture and PCRIS6110 to be 20%, 50.34% and 77.24%, respectively.[17] PCR targeting IS6110 could detect 22.6% and 59.21% additional cases when compared to MGIT culture and ZN microscopy, respectively, in our study.

The diagnosis of extrapulmonary tuberculosis (EPTB) still remains a challenge in children. Our study has shown 65.2% positivity of PCR IS6110 in EPTB specimens. Another Indian study has also reported 63% positivity of PCR targeting IS6110 element in EPTB clinical specimens.[18] The sensitivity of IS6110 PCR was higher in extrapulmonary samples as compared to MGIT culture (43.5%) and microscopy (5.4%) in our study as also reported in another study showing sensitivity of PCR to be 70% and that of MGIT and ZN microscopy to be 15% and 5%, respectively, in extrapulmonary specimens.[19] Most of the EPTB cases were tubercular meningitis (69.5%). Studies conducted by Amin et al.,[20] Siddique et al.[19] reported 42.1% and 36% positivity of PCR IS6110 in CSF, respectively, which was much lower than that of our study showing positivity of PCR to be 64.1% in CSF. Ascitic fluid and pleural fluid showed higher sensitivity in our study as compared to another study showing sensitivity in pleural fluid and ascetic fluid to be 22% and 27%, respectively[21] probably due to small number of these specimens in the study.

The limitation of PCR assays is the availability of laboratory facilities for PCR with least possible contamination.

6. Conclusion

PCR targeting IS6110 has high specificity, and sensitivity (100%, 69.01%) in diagnosis of TB in children. It has good diagnostic efficacy both in pulmonary and extrapulmonary forms of TB showing 76% and 65.2% sensitivity. The present study also revealed that PCR using IS6110 had better efficacy than conventional microscopy and MGIT960 culture system in diagnosing TB in children.

Author contributions

RD and DA designed the study, HP and SF helped in data collection, RB, MK, RD and DA contributed in data analysis, drafting the article and final approval.

Conflicts of interest

The authors have none to declare.

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Original article

Smoked and smokeless tobacco use among pulmonary tuberculosis patients under RNTCP in urban Puducherry, India

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ABSTRACT

Background: Smoking is associated with unfavourable treatment outcomes like failures and defaults among the TB patients.

Objectives: To study the prevalence and pattern of tobacco use among the pulmonary tuberculosis (PTB) patients in urban Pondicherry and study the association of various socio-demographic variables with current smoked and smokeless tobacco users.

Methods: A cross-sectional study was conducted among 235 PTB patients from 6 randomly selected urban PHCs of Pondicherry from Jan 2013 to March 2014. Fagerstrom Test for Nicotine Dependence was used. Chi-square test and multiple-logistic regression were done.

Results: Prevalence of smoking among the PTB patients at the time of TB diagnosis was 35.3%, whereas the same during the continuation phase (CP) was 23.4%. Among 83 smokers at the time of diagnosis, 52 modified and 31 did not modify their smoking after TB diagnosis. Similarly, prevalence of smokeless tobacco use both at the time of TB diagnosis and during CP was 9.8%. Male and lower education level was associated with current smoking. Similarly, female and lower education level was associated with current smokeless tobacco use.

Conclusions: One-third of PTB patients used smoked or smokeless tobacco during their CP. Health programme needs to concentrate on PTB patients who continue to use smoked or smokeless tobacco during their treatment; necessary interventions need to be planned.

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1. Introduction

Tuberculosis continues to be a major public health problem around the world.[1] India is also one of the high TB burden countries in the world. In 2012, out of the estimated global annual incidence of 86 lakhs TB cases, 23 lakhs were estimated to have occurred in India. As per World Health Organization (WHO) estimated burden of tuberculosis in India (2012), the incidence was 176 per lakh and prevalence was 230 per lakh populations.[1]

India is positioned second in terms of tobacco consumption in the world.[2] According to Global Adult Tobacco Survey (GATS) in 2009–2010, 25% of the adults were smokeless tobacco users and 14% were current smokers.[3] Several studies have demonstrated that smoking is positively associated with TB incidence.[4,5] Three recent systemic reviews and meta-analysis have confirmed that smoking is an important risk factor for being infected with Mycobacterium Tuberculosis, progression as a clinical disease and dying from TB.[6–8] In India too studies have shown that, smoking is a risk factor for tuberculosis disease. Heavy smoking leads to more risk, the odds ratios for mild (1 ± 10 cigarettes/day), moderate (11 ± 20/day), and heavy (>20/day) smokers were 1.75, 3.17, and 3.68, respectively.[4] Tobacco smoking is significantly associated adverse treatment outcomes like treatment failures and defaults among the TB patients.[9–11] Relapse rate were significantly associated with smokers by the study done in South India (AOR 3.1).[9] Smoking is the independent risk factor for poor treatment outcome.[12] One in every five deaths due to TB could be prevented if the patients were not smoking.[13–15] In Malaysia, Awaisu et al.[16] found that the prevalence of current smoking among TB patients was 40%. The same study also reported that the current TB smokers were either moderately dependent (51%) or highly dependent (27%) to nicotine. In India, there are few studies on prevalence of tobacco use among TB patients and their dependence to nicotine.

Screening for Nicotine dependence among TB patients is necessary to assist Revised National Tuberculosis Control Programme (RNTCP) in developing effective interventions to deal with the problems related to smoking. In the present study, Fagerstrom Test for Nicotine Dependence (FTND) was used as a screening tool for assessing the severity of tobacco use among the pulmonary tuberculosis (PTB) patients.

The objectives of the study were to assess the prevalence and pattern of tobacco use among the PTB patients residing in urban Pondicherry and study the association of various socio-demographic factors with current tobacco smoking and current smokeless tobacco use.

2. Materials and methods

This was a community-based cross-sectional study carried out between January 2013 and March 2014 in Pondicherry district, India. The total population of Pondicherry is 1,244,464[17] and around 68% of population in Pondicherry live in urban areas.

The sample size was calculated to be 235 assuming that the prevalence of smokeless tobacco use among tuberculosis patients was 11%,[18] absolute precision of 4% and 95% confidence interval. The study was restricted to urban PHCs of Pondicherry. Six out of twelve urban PHCs[19] were randomly selected till the cumulative total of PTB patients of previous year satisfied the sample size necessary for the study. All the eligible consecutive PTB patients from the selected six PHCs were included in the study. The location of these selected PHCs were scattered all over urban Pondicherry, thus it is expected that the TB patients selected from these PHCs will represent the total PTB patients from urban Pondicherry. PTB patients aged 15 years and above were included in the study. Category IV tuberculosis patients were excluded. Eligible patients were interviewed during the continuation phase (CP) of their TB treatment.

The study was approved by the Institute Ethics Committee. Demographic details of eligible PTB patients were obtained from the TB treatment cards maintained for each patient in their respective PHCs. All the eligible TB patients were contacted at their place of residence or at the health centres as per their convenience. Adequate time was spent with each PTB patient to develop rapport with them following which informed consent was taken from them. The houses which were either locked or where the patients were not present at the time of the visit were revisited one more time at a later date. Patient who could not be contacted during the 2nd visit were not contacted further.

2.1. Study tools

A pre-tested interview schedule was used to collect information from the study participants. Socio-demographic factors like age, gender, education, occupation were obtained from the subject by personal interview. Education status was classified based on the Tamil Nadu education board. Socio-economic status was classified using Modified Kuppusamy classification – 2012 based on income, education and occupation.[20] Occupation was classified using National Classification of Occupations, 2004. The definitions for smoked and smokeless tobacco users as followed in the GATS India[3] were used in the present study.

2.2. FTND (Fagerstrom Test for Nicotine Dependence)[21]

There are separate scales available for assessing smoked and smokeless tobacco use. These scales were used in the present study to assess the level of nicotine dependence among PTB patients. There were 6 items in the scales. These scales are used for both current smoker and smokeless tobacco users. The various item scores are summed to yield a total score which ranges between 0–10. A score ranging between 0–3, 4–6 and 7–10 were considered minimally dependent, moderately dependent and highly dependent respectively.

Apart from this the PTB patients were also asked if they had quit, reduced, increased or maintained their Smoke and Smokeless tobacco use after their TB diagnosis.

2.3. Data analysis

Data collected was entered in the Epi-data v3.1.[22] it was exported and analysed using IBM SPSS v20.[23] Description of all

socio-demographic variables and tobacco prevalence among PTB patients is reported in percentages. Pattern of tobacco use and nicotine dependence are also reported in percentages. Association between socio-demographic variables with tobacco use was analysed using Chi-square test. Multiple binary logistic regression was used to find out the independent predictors for tobacco use among PTB patients and adjusted odds ratio was calculated.

3. Results

Out of the total 265 PTB patients registered in the select six PHCs, 235 (88.7%) were included and the rest 30 (11.3%) could not be contacted. Among the 30 PTB patients, 11 (4.6%) could not be contacted even after two home visits, 15 (5.6%) had shifted their residence and 4 (1.5%) had died. The mean (SD)

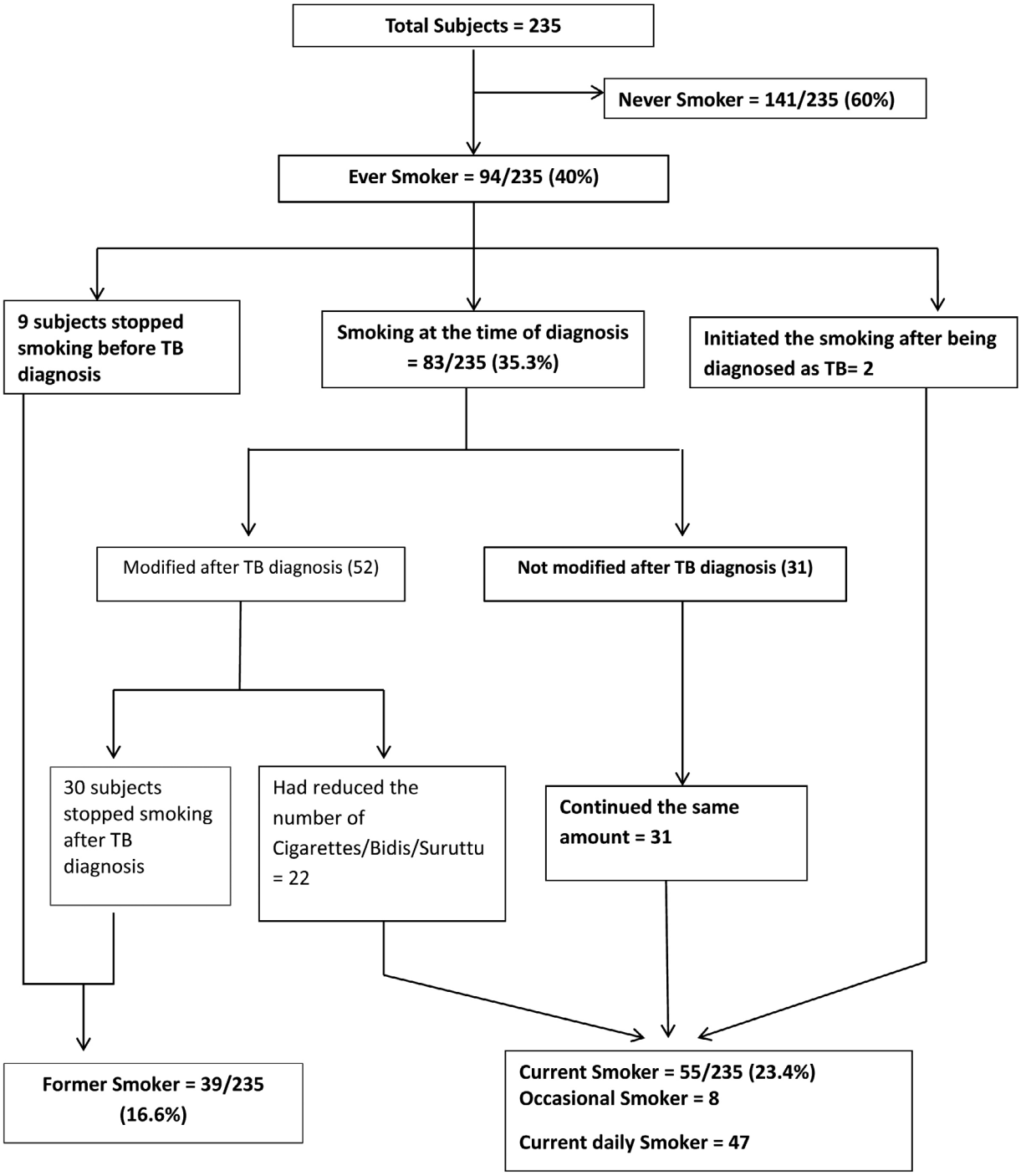

Fig. 1 – Smoked tobacco use among PTB patients in urban PHCs.

time interval between initiation of CP of TB treatment and data collection was 12 ± 4 weeks.

3.1. *Socio-demographic details*

Majority of the PTB patients were males (79.6%) and their Mean (SD) age was 44 ± 14 years. Majority (44%) of the PTB patients had received education up to Upper primary/Secondary class (Class 6–10), 28.5% had no formal education/were educated up to Primary class (Class 1 to 5) and rest 27.5% were educated beyond class 10 (Higher secondary/Graduate). Almost 70% of them were working as unskilled/semiskilled/skilled jobs, 13% were unemployed and rest 17% were working as Professionals/Businessmen. Nearly half (49%) of them belonged to lower SES. More than three-fourth of PTB patients received Cat I (77%) treatment and the rest received Cat II regimen.

Figure 2: Smokeless tobacco use among PTB patients in Urban PHCs

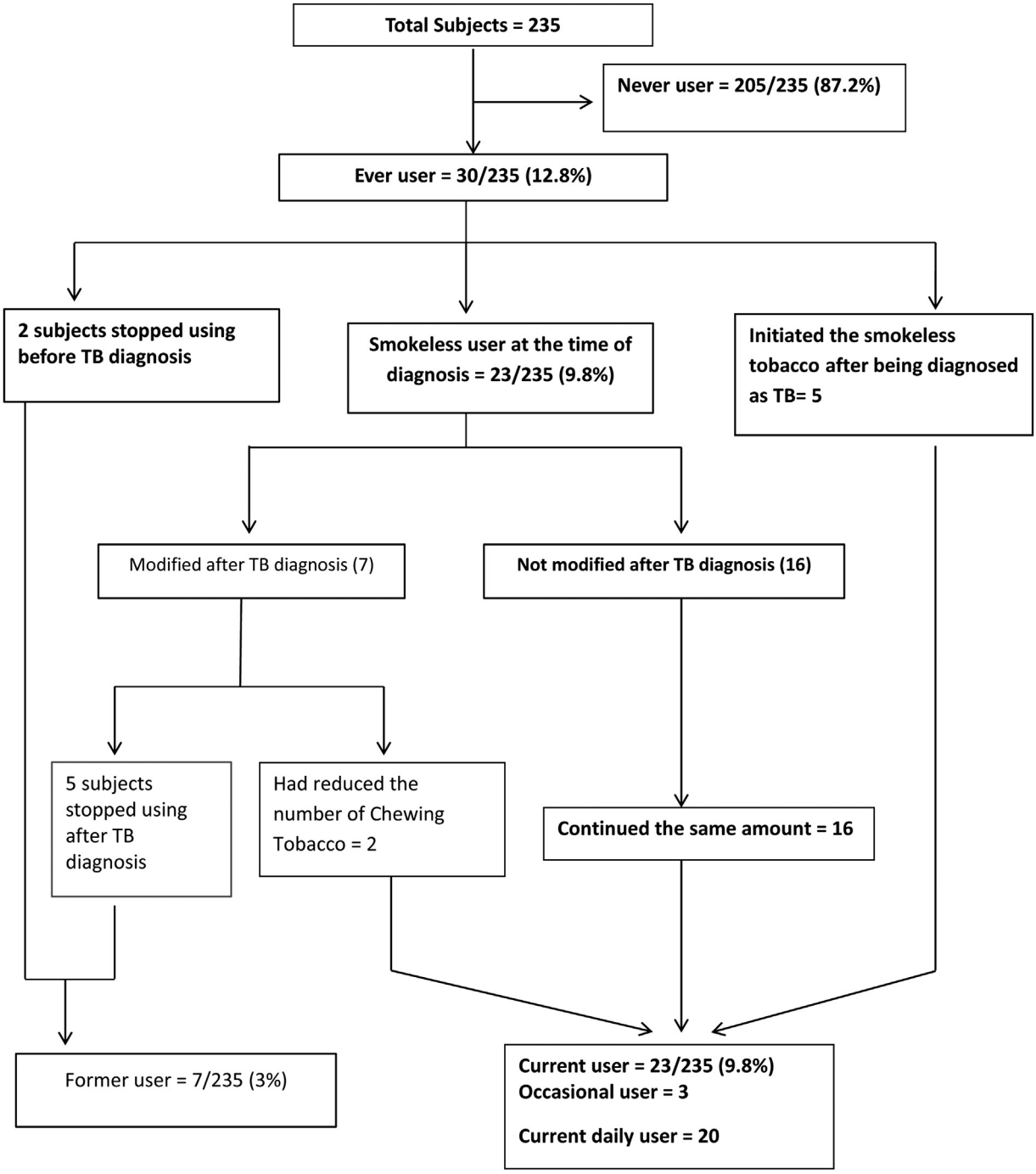

Fig. 2 – Smokeless tobacco use among PTB patients in urban PHCs.

3.2. Smoked tobacco status

Out of the total 235 PTB patients, 94 (40%) were ever smokers. A total of nine patients had quit smoking before their TB diagnosis and two initiated smoking after being diagnosed as TB patient. Thus the prevalence of smoking at the time of diagnosis was 35.3% ($n = 83$) (Fig. 1). Out of the total 83 smokers at the time of diagnosis, 52 (62.7%) PTB patients modified (30 patients quit smoking and 22 patients reduced) their smoking status after being diagnosed as TB and the rest 31 (37.3%) maintained their smoking status without modifying their behaviour.

The prevalence of Former smokers was 16.6% ($n = 39$). Nine patients had quit their smoking before TB diagnosis and rest 30 patients had quit smoking after their TB diagnosis. There were 31 patients who could not modify, two patients initiated smoking and 22 had reduced their smoking behaviour after being diagnosed as TB, totally 55 (23.5%) were current smokers during treatment. Among the current smokers eight were Occasional smokers and 47 were current daily smokers.

3.3. Smokeless tobacco status

The prevalence of ever smokeless tobacco user was 12.8% (30). The prevalence of smokeless tobacco use both at the time of diagnosis and in the CP, was 9.8% ($n = 23$) (Fig. 2).

Among the 23 PTB patients who were smokeless tobacco users at the time of diagnosis, 7 modified (5 quitted and 2 reduced) their behaviour and the rest 16 continued to consume smokeless tobacco as they were taking before their TB diagnosis.

Among the 23 current user during treatment, 20 (87%) were daily user and rest 3 (13%) were occasional users.

3.4. Association between socio-demographic variables with current smokers during treatment

Univariate analysis showed that PTB patients in the age group of 30–59 years, Male gender, having low education (No formal education/Primary class and Upper primary/Secondary class), who were unemployed or were involved in unskilled/semi-skilled/skilled jobs, belonging to lower SES and patients on Cat II regimen were more likely to be currently smoking during TB treatment. These variables were subject to multivariate logistic regression by using enter method which showed that male patients and having lower education were significantly associated with current smoking during TB treatment (Table 1).

3.5. Association between socio-demographic variables with current smokeless tobacco users during treatment

Univariate analysis showed that female patients, having low education (No formal education/Primary class), Lower SES were significantly associated with current smokeless users during TB treatment. These variables were subjected to multivariate logistic regression by using enter method which showed that female gender and patients having lower education (No formal education/Primary class) were significantly associated with current smokeless tobacco use during TB treatment (Table 2).

Table 1 – Association between socio-demographic variables with *current smokers among* PTB patients from urban Pondicherry.

Variables	Total (%) N = 235	Current smoker (%) N = 55	Current non-smoker (%) N = 180	OR (CI)	P value	AOR (CI)	P value
Age group in years							
15–29	41 (100)	3 (7.3)	38 (92.7)	1		1	
30–44	81 (100)	19 (23.5)	62 (76.5)	3.9 (1.1–14)	0.04*	0.86 (0.2–3.7)	0.84
45–59	80 (100)	26 (32.5)	54 (67.5)	6.1 (1.7–21.6)	0.005*	1.4 (0.34–6.0)	0.62
≥60	33 (100)	7 (21.2)	26 (78.8)	3.4 (0.8–14.4)	0.09	0.64 (0.12–3.5)	0.62
Gender							
Male	187 (100)	54 (29)	133 (71)	19.1 (2.5–141.8)	0.004*	15.4 (1.9–122.2)	0.009*
Female	48 (1000)	1 (2.1)	47 (97.9)	1		1	
Education status							
No formal education/Primary class	67 (100)	23 (34.3)	44 (65.7)	7.9 (2.6–24.7)	<0.001*	6.8 (1.4–33.9)	0.02*
Upper primary/Secondary class	103(100)	28 (27.2)	75 (72.8)	5.7 (1.9–17.1)	0.002*	4.3 (0.9–18.7)	0.053
Higher secondary/Graduate	65 (100)	4 (6.2)	61 (93.8)	1			
Occupational status							
Unemployed	32 (100)	10 (31.2)	22 (68.8)	5.6 (1.4–22.6)	0.015*	1.3 (0.2–8.3)	0.82
Unskilled/Semiskilled/Skilled	163 (100)	42 (25.8)	121 (74.2)	4.3 (1.3–14.6)	0.02*	0.97 (0.2–5.1)	0.97
Professional/Businessman	40 (100)	3 (7.5)	37 (92.5)	1		1	
Socio-economic status							
Upper and Middle class	120(100)	19 (15.8)	101 (84.2)	1		1	
Lower class	115(100)	36 (31.3)	79 (68.7)	2.4 (1.3–4.5)	0.006*	1.2 (0.6–2.7)	0.6
Category							
Cat I	181 (100)	89 (65.4)	92 (34.6)	1		1	
Cat II	54 (100)	25 (49.0)	29 (51.0)	2.5 (1.3–4.8)	0.008*	1.8 (0.9–3.8)	0.11

OR – odds ratio, AOR – adjusted odds ratio, CI – confidence interval, * – significant.

Table 2 – Association between socio-demographic variables with *current smokeless users* among PTB patients from urban Pondicherry.

Variables	Total (%) N = 235	Current user (%) N = 23	Current non-user (%) N = 212	OR (CI)	P value	AOR (CI)	P value
Age group in years							
15–29	41 (100)	2 (5)	39 (95.1)	1		Not included	
30–44	81 (100)	6 (7.4)	75 (92.6)	1.6 (0.3–8.1)	0.6		
45–59	80 (100)	9 (11.2)	71 (88.8)	2.5 (0.5–12)	0.26		
≥60	33 (100)	6 (18.2)	27 (81.8)	4.3 (0.8–23.1)	0.09		
Gender							
Male	187 (100)	14 (7.5)	173 (95.5)	1		1	
Female	48 (1000)	9 (18.8)	39 (81.2)	2.9 (1.2–7.1)	0.023*	3.5 (1.3–9.5)	0.012*
Education status							
No formal education/Primary class	67 (100)	14 (21)	53 (79)	5.5 (1.5–20)	0.01*	4.6 (1.1–20)	0.04*
Upper primary/Secondary class	103(100)	6 (5.8)	97 (94.2)	1.3 (0.3–5.3)	0.74	1.2 (0.3–5.7)	0.81
Higher secondary/Graduate	65 (100)	3 (4.6)	62 (90.2)	1			
Occupational status							
Unemployed	32 (100)	6 (18.8)	26 (81.2)	Not applicable for any test		Not applicable	
Unskilled/Semiskilled/Skilled	163 (100)	17 (10.4)	146 (89.6)				
Professional/Businessman	40 (100)	0 (0)	40 (100)				
Socio-economic status							
Upper and Middle class	120(100)	6 (5)	114 (95)	1		1	
Lower class	115(100)	17 (14.8)	98 (85.2)	3.3 (1.3–8.7)	0.016*	2.3 (0.77–6.6)	0.14
Category							
Cat I	181 (100)	17 (9.4)	164 (90.6)	1		Not included	
Cat II	54 (100)	6 (11)	48 (89)	1.2 (0.5–3.2)	0.71		

OR – odds ratio, AOR – adjusted odds ratio, CI – confidence interval, * – significant.

3.6. *Pattern of current smokers and current smokeless users*

The predominant substances used by the current smokers were bidis, cigarettes and suruttu in 58.2%, 38.2% and 3.6% respectively. Around 78% (*n* = 43) were smoking ≤10 cigarettes/bidis/suruttu per day and the rest 22% (*n* = 12) were smoking 11–20 cigarettes/bidis/suruttu per day. Three-fourth (*n* = 42) were minimally dependent and 13 (23.6%) were moderately dependent on nicotine. No one was highly dependent to nicotine (Table 3).

The predominant substance used by the current smokeless tobacco users were Betel tobacco leaves, Pan Masala, Khaini (Hans) and Gutkha in 34.7%, 30.4%, 21.7%, and 13.0% respectively. Four patients were using one Pouch and nineteen were using 2–4 pouches per day, sixteen patients were minimally dependent, six were moderately dependent and only one patient was highly dependent to nicotine (Table 3).

3.7. *Treatment outcome*

The overall cure, completion, default, failure and death rate were 73.2%, 12.3%, 6.8%, 5.1% and 2.6% respectively. The failure and death rate were comparatively more among the current smokers as compared to the non-smokers. The cure and

Table 3 – Pattern of current smokers and current smokeless users among PTB patients of Pondicherry.

Current smokers, N = 55 (100)		
Type of smoking substance (%)	No of cigarettes/bidis/suruttu per day (%)	Dependence level (0–10) – N (%)
I. Bidi – 32 (58.2) II. Cigarette – 21 (38.2) III. Suruttu – 2 (3.6)	≤10 Number – 43 (78.2) 11–20 Number – 12 (21.8)	Minimally dependent (0–3) – 42 (76.4) Moderately dependent (4–6) – 13 (23.6) Highly dependent (7–10) – 0 (0)
Current smokeless users, N = 23 (100)		
Type of tobacco substance	No. of pouches per day	Dependence level
I. Betel tobacco leaves – 8 (34.7) II. Pan Masala – 7 (30.4) III. Khaini (Hans) – 5 (21.7) IV. Gutkha – 3 (13.0)	One pouch – 4 (17.4) 2–3 in number – 18 (78.3) 4 or more in number – 1(4.3)	Minimally dependent (0–3) – 16 (69.6) Moderately dependent (4–6) – 6 (26.1) Highly dependent (7–10) – 1 (4.3)

Table 4 – Treatment outcome among current smokers in urban PTB patients of Pondicherry.

Treatment outcome	Current smoker – N (%)	Current non-smoker – N (%)	Total – N (%)
Cured/completed	41 (74.6)	160 (88.9)	201 (85.5)
Default	4 (7.3)	12 (6.7)	16 (6.8)
Failure	7 (12.6)	5 (2.7)	12 (5.1)
Died	3 (5.5)	3 (1.7)	6 (2.6)
Total	55 (100)	180 (100)	235 (100)

default rate were similar among both current smokers and non-smokers (Table 4).

4. Discussion

In the present study, 35.3% were smoking at the time of TB diagnosis, whereas only 23.4% were smoking during the CP. This decrease in smoking status could be because of the counselling sessions inbuilt in the delivery of RNTCP services. Among the 35.3% patients who smoked at the time of diagnosis – 37% continued to smoke the same amount, 36.5% stopped smoking and rest 26.5% reduced their smoking use after their TB diagnosis. There were even 2 PTB patients who initiated smoking after TB diagnosis.

Similarly among the 23 (9.8%) current smokeless tobacco users during the CP, 16 patients continued to take the same amount, 2 patients had decreased and 5 patients had initiated using smokeless tobacco after TB diagnosis. This reflects that necessary health advice against the use of nicotine in form of smokeless tobacco was also deficient. Training of health personnel for the easy identification of these resistant cases in the field is necessary for ensuring appropriate interventions.

Among the current smokers during treatment, 24% of patients were moderately dependent to nicotine. Among current smokeless tobacco users, 26% were moderately dependent and 4% were highly dependent to nicotine. These patients need to be referred to the psychiatrist for diagnostic evaluation and nicotine replacement therapy.

Prevalence of smoking at the time of diagnosis was comparable to the studies done by Kolappan et al.[5] and Rao et al.[24] where the same was 46.4% and 48.1% respectively. In the present study, 76% were minimally dependent and 24% were moderately dependent to Nicotine. However, a study done in Malaysia by Awaisu et al.[16] found that 21% were minimally dependent, 51% were moderately dependent and 27.5% were highly dependent to nicotine. Reasons could be due to the study by Awaisu et al.[16] assessed the nicotine dependence at the time of diagnosis whereas the present study assessed during the CP.

In the present study predominant substances used for smoking were bidis (58%) and cigarettes (38%). This finding was comparable to a study from Kerala by Pradeepkumar et al.[18] who reported that bidis were the used by majority of the TB patients maximum (70%). In the present study, 78% were smoking ≤10 cigarettes or bidis per day, and 22% were smoking 11–20 cigarettes/bidis per day. This was in contrast to a study done by Kolappan et al.[4] where 39% were smoking ≤10 cigarettes or bidis per day, 33% were smoking 11–20 cigarettes/bidis per day and the rest 28% were smoking more than 20 cigarettes/bidis per day. This difference could be due to the fact that the timing of evaluation of assessment of smoking status by Kolappan et al.[4] was assessed at the time of TB diagnosis whereas the timing of evaluation in the present study was different.

In the present study, prevalence of smokeless tobacco use at the time of diagnosis was 9.8% and during the CP was also 9.8% whereas the study from done in Karnataka by Deepak et al.[25] reported found that smokeless tobacco use during diagnosis was 40%. This difference could be due to the fact that smokeless tobacco use among in general population is more in Karnataka as compared to Pondicherry (GATS survey 2009–2010).[3]

In the present study, among male TB patients 29% of male TB patients were current smokers during treatment. This was comparable to a study done in from Kerala by Pradeepkumar et al.[18] who reported that 27% of the male TB patients were current smokers during treatment. In the present study it was found that male gender and, lower education status were associated with current smoked tobacco use during treatment. Similarly in the present study, and female gender and lower education level was associated with current smokeless tobacco use during treatment. Necessary health interventions should be targeted taken in under RNTCP for the TB patients with these characteristics.

4.1. *Strengths*

Almost 89% of the PTB patients in the selected 6 PHCs of Pondicherry were covered in the present study. The location of these 6 selected PHCs were scattered all over the urban areas of Pondicherry. It is thus expected that patients selected from these selected PHCs will be representative of the total urban PTB patients of Pondicherry. Thus generalization of the present study findings may be possible can be done to all PTB patients in urban Pondicherry. This is one of the few studies where which assessed the prevalence of tobacco use is assessed both at the time of TB diagnosis and during the CP of TB treatment. It is thus possible to quantify the changes in smoking status pattern of among PTB patients between at the time of diagnosis and during CP of TB treatment.

4.2. *Limitations*

The present study, participants were limited to the PTB patients attending government health facilities. Thus the findings from the present study is thus only are applicable only to PTB patients attending government health facilities. Another likely bias due to self-reported on tobacco use among by the study participants cannot be ruled out.

5. Conclusion

The present study found that 40% of PTB patients were smokers at the time of their TB diagnosis and 23.4% were current smokers during the CP. Similarly, 10% of PTB patients were smokeless tobacco users at the time of diagnosis and same proportion were too current smokeless users during the CP. The national TB programme (RNTCP) need to plan appropriate interventions Health programme needs to concentrate for the PTB patients who continued to use smoked or smokeless tobacco during their TB treatment; necessary interventions need to be planned for these patients. There were few PTB patients who even initiated smoked or smokeless tobacco use after their TB diagnosis. Health workers need to be trained to screen for nicotine dependence at the field for identifying and facilitating appropriate interventions. These interventions targeting tobacco use are also expected to improve TB the treatment compliance and outcome of TB.

Conflicts of interest

The authors have none to declare.

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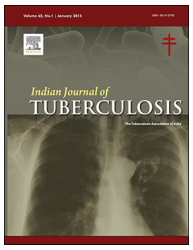

Original Article

Tuberculosis prevalence and socio-economic differentials in the slums of four metropolitan cities of India

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ABSTRACT

Aim: To understand tuberculosis (TB) prevalence among the slum dwellers of metropolitan cities of India and the factors associated with TB prevalence.
Methods: National Family Health Survey-III data for four metropolitan cities namely, Delhi, Mumbai, Kolkata and Chennai was used for this study.
Results: Prevalence of TB is significantly ($P = 0.001$) higher in the slums than non-slums of Mumbai, Chennai and Kolkata cities. As the living standard increases, TB prevalence decreases. Logistic regression analysis uncovers that lower standard of living is highly associated with TB followed by place of residence (slum or non-slum).
Conclusion: Mumbai has the highest prevalence among the four cities studied herein. Living standards, place of residence and absence of windows and electricity in the households are the factors associated with TB prevalence.

1. Introduction

Tuberculosis (TB) continues to be a huge peril disease against the human population; WHO documents that after HIV/AIDS, TB (*Mycobacterium tuberculosis*) is a major killer of the human population. In 2014, 9.6 million people are estimated to have fallen ill with TB and 1.4 million deaths occurred among HIV-negative TB patients.[1] With the support of WHO, the Government of India's target specific approach such as RNTCP resulted in reduction of TB mortality to a great extant. TB has an important place in Millennium Development Goals; it is mentioned in the following as: in Goal 6 – to combat HIV/AIDS, malaria and other diseases; in Target 8 – to have halted by 2015 and begun to reverse the incidence of malaria and other major diseases, including TB; in Indicator 23 – between 1990 and 2015, to halve the prevalence and death rates associated with TB.[2] India has highest annual TB incidence; globally, one-fifth of TB cases are from India and it is estimated that about 40% of Indian population is infected with TB bacillus. In India, premature death due to TB is greater than 80% of the burden of disease as revealed in terms of disability-adjusted life years (DALYs) lost.[3]

TB is more common among the low socio-economic section of the population and marginalised sections of the community.[4] Factors influencing communicable diseases in slums are poverty, uncontrolled migration, overcrowding, rapidly depleting natural resources and poor water management.[5] Incessant growth of the slum population is not only posing a herculean task for civic authorities, but also for the public health personnel. Morbidity prevalence is more in the slum areas than in the rural areas. From the literatures it is well

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known that communicable diseases' prevalence is more in the slum areas; recent studies demonstrate that non-communicable diseases are also on the rise in slum areas.[6]

The health hazards of the urban slum dwellers are mainly due to poverty, malnutrition and contaminated environment where they live. In such an environment, slum dwellers are exposed to all types of communicable diseases including TB, and endless malnutrition resulted in higher rates of morbidity and mortality in the slums areas. With the available limited daily wages, slum dwellers are helpless to think of nutritional/balanced diet for their family. Small pox disease has been eradicated, and recently, polio has also been declared as eradicated from India. Slum dwellings do not have adequate sunlight exposures; no ventilation, no drainage inadequate sanitation, poor solid waste management and limited access to healthcare services are the factors that play a central role for the high disease prevalence.[7] As a result of heavy migration to the major cities, slum population growth rate is more than the India's population growth rate, and slums are rapidly growing like mushrooms. Studies on TB prevalence among slum children are available, but comparison of TB prevalence simultaneously among slum and non-slum children is seldom available. Moreover, the highest proportion of the slum population of India is dwelling in the four metropolitan cities, that is, Delhi, Mumbai, Kolkata and Chennai. Hence, in this paper, it is attempted to gauge TB prevalence among the slum and non-slum dwellers in metropolitan cities, namely Delhi, Mumbai, Kolkata and Chennai and to examine the factors associated with TB prevalence.

2. Material and methods

National Family Health Survey-III (NFHS-III) data for the four metropolitan cities namely, Delhi, Mumbai, Kolkata and Chennai was used for the analysis. These data were obtained from *MeasuresDhs*, *US*.[8] The households were classified as slum and non-slum by two agencies, viz., Census of India and NFHS-III, and were only considered for analysis. As per Census of India, 19,257 individuals were classified from slum households in the four cities. NFHS-III interviewing team supervisor at the time of the fieldwork had classified 19,593 individuals from slum households in these four cities. There were 15,099 individuals who were commonly identified from slum households by both census and NFHS-III agencies. Hence, these 15,099 individuals were considered for further analysis as slum samples. With the same principle, non-slum individuals (18,751) were considered for the analysis from the four cities for this study. NFHS-III included 8 mega and medium cities of India for collecting slum and non-slum data. About 1000 sample households of slums and 1000 households for non-slums were the target from each Primary Sampling Unit (wards) of city and the detailed sampling plan is available in NFHS-III slum report.[9] Standard of Living Index (SLI) is a proxy measure for determining the socio-economic condition of a household.[10] Scores are given by examining/interviewing a household in terms of ownership of household goods by adding these scores; total of SLI is obtained. SLI is classified as 0–14 low, 15–24 medium and 25+ as high categories. Few variables that are more relevant for TB prevalence are namely,

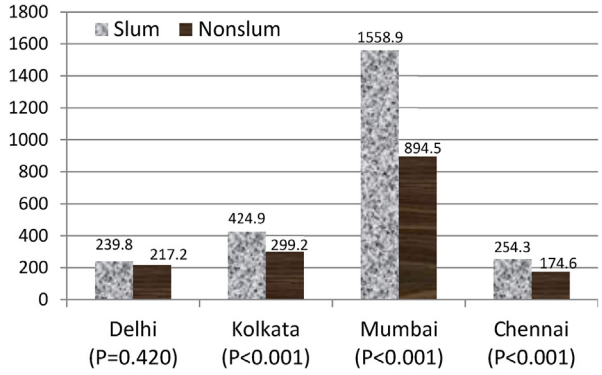

Fig. 1 – TB prevalence per 100,000 population in the four metropolitan cities by place of residence.

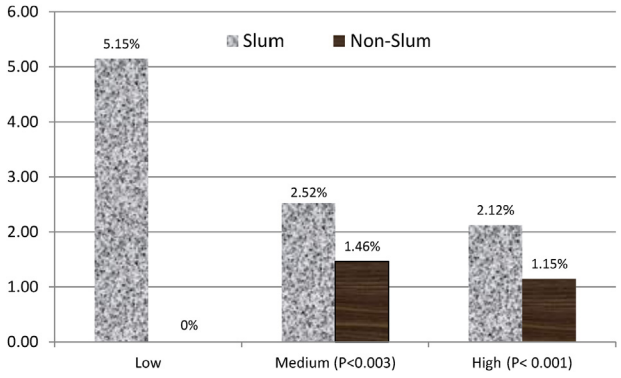

Fig. 2 – TB prevalence per 100 population in the four metropolitan cities by Standard of Living Index.

any household member suffered from TB, any treatment was done for drinking water, presence of window, electricity in the households, place of residence (slum or non-slum) and SLI, and these are considered for this study.

TB prevalence (for Figs. 1 and 2) for slum and non-slum areas was adjusted with weights for the respective areas. Weights were calculated from census data[11] for slum and non-slum populations of the four cities; weight is total number of samples covered by NFHS-III to the census population.

Chi-square test was applied to compare the proportions of SLI between slum and non-slum areas among the four cities and multivariate logistic regression analysis was performed. Data were analysed using SPSS 20.0 Statistical package.

3. Results

Among 33,850 individuals considered for analysis, 457 and 274 individuals reported that they had suffered from TB from slum and non-slum areas, respectively.

The percentage distribution of TB prevalence is depicted in Fig. 1. The difference in TB prevalence between slum and non-slum is high and it is statistically significant ($P < 0.001$) in each city, that is in Mumbai, Kolkata and Chennai and the exception is Delhi. Among the four cities, Chennai non-slum dwellings

Table 1 – TB prevalence in the four metropolitan cities by Standard of Living Index and place of residence.

		Low		Medium		High	
		Slum	Non-slum	Slum	Non-slum	Slum	Non-slum
Delhi	Total	232	140	1706	614	2704	7513
	Suffered from TB	4.74%	0%	1.64%	1.30%	0.92%	1.30%
Kolkata	Total	321	65	1832	769	2003	3752
	Suffered from TB	1.87%	0%	3.88%	3.51%	2.75%	0.43%
Mumbai	Total	63	13	1184	180	2626	2981
	Suffered from TB	19.05%	0%	3.46%	0%	4.15%	2.55%
Chennai	Total	307	60	1139	406	982	2258
	Suffered from TB	9.45%	0%	3.16%	0%	1.63%	1.06%

Table 2 – Results of logistic regression analysis for TB prevalence.

	B	SE	Wald	df	P	OR	95%CI
Electricity	−0.643	0.193	11.078	1	0.001	0.526	0.360–0.768
Any treatment for water	−0.191	0.078	5.983	1	0.014	0.826	0.708–0.963
Window	−0.750	0.131	32.726	1	0.000	0.472	0.365–0.611
Slum or non-slum	0.859	0.080	115.144	1	0.000	2.360	2.017–2.760
SLI	1.372	0.175	61.562	1	0.000	3.944	2.799–5.556
Constant	−4.335	0.069	3961.711	1	0.000	0.013	

have least TB prevalence, followed by Kolkata. Mumbai has the highest prevalence of TB in both slum and non-slum areas.

Fig. 2 is presented for TB prevalence by the SLI. It is evident from this figure that as SLI changes to higher level, there is decline in TB prevalence among the slum dwellers. The same trend is observed for non-slum dwellers also. The proportions difference of TB prevalence between slum and non-slum is statistically significant ($P < 0.002$) among the medium and high categories of SLI. From this figure, one can assume that slum people classified as low SLI may have about double the magnitude for suffering from TB than the people classified as medium or high SLI categories.

Table 1 presents TB prevalence in the four metropolitan cities by the SLI. As depicted in Fig. 2, there is no TB prevalence among the non-slum dweller belonging to low category of SLI. As SLI changes to higher levels, TB prevalence comes down in all the four cites. About 50% of the non-slum category cells have zero value and hence chi-square test was not carried out.

Table 2 is presented for the multivariate binary logistic regression analysis to understand the relative importance of each variable and their contribution in this binary logistic regression analysis for TB prevalence. Dependent variable is 'suffered from TB: yes or no (Yes = 1 and No = 0)' and the response 'yes' is considered as risk group. From this logistic regression analysis, it is evident that persons in the low SLI is having about 4 times higher risk than those who are either medium or high categories. Followed by dwelling in slum and non-slum areas, odds ratio (OR) for the slum dwellers is approximately 2.4 times higher risk of having TB when compared with non-slum population. If a household is not having window, its risk of infection with TB is approximately two times more than the household having window, and the similar interpretation holds good for households not possessing electricity. Not treating the water before drinking has no/minimum risk with OR being 1.25.

In logistic regression analysis, the dependent variable's reference category is 'no' for TB infection. In independent variables, the reference categories are as follows: SLI: medium or high categories, place of residence: non-slum area, not having window, not having electricity and water treated for drinking purpose. Conditional backward elimination method was used for the variable selection; all the variables supplied are included in the model except for 'Type of household'. All the variables significantly (maximum $P = 0.014$) contributed to the model. Confidence interval (CI) is another measure used to gauge the OR, and it may be noted that none of the 95%CI include the value one, indicating that the estimated OR is stable and cannot be regarded as not associated with reference group.

4. Discussion

A study conducted in Delhi slums by Saha et al.[12] revealed that in lower socio-economic strata TB continued to be the major cause of death; they have also shown that about one-fourth of the mortality are due to TB in Delhi slums. One of the important measures of prevention for any disease is to educate the public about mode of transition and symptoms of the disease. A study conducted in the South Indian slums by Chinnakali et al.[13] has revealed that 94% of slum dwellers had heard about TB and 82% of them knew about symptoms of TB.

It is reported that TB prevalence is 5.4 and 3.8 per 1000 population for male and females, respectively; it is also shown in Ahmedabad city that higher risk is associated with old age slum population.[14] There is 2.91% (unweight) prevalence in the slum population of all four cities in this study.

In a cross-sectional survey conducted in South India, it is found that SLI and TB prevalence are in the inverse association, low category of SLI and the prevalence was

significantly high with the rest of the categories. It is also shown that people living in the Katcha houses had reported significantly more TB prevalence than those who are living in other types of houses.[15] In this paper, it is found that TB prevalence is significantly associated with place of residence (slum and non-slum) in all the four cities (Fig. 1) and it is associated with SLI categories (Fig. 2), that is levels of SLI and TB prevalence is inversely associated.

Association between housing condition and TB prevalence is studied by Wanti et al.[16] using a cross-sectional case–control study. They have demonstrated that floor of the house, home ventilation, natural lighting, the temperature of the house, home humidity and population density are related with the incidence of TB. In this paper, factors associated with reported TB prevalence was studied, and presence of window was found as one of the factor associated with TB.

As like any other secondary data, here too some limitations as the following were encountered. The first one is that TB prevalence is not significantly different between slum and non-slum dwellers in Delhi. This situation might be as result of ambiguity in classifying households as slum and non-slum by the two agencies, that is Census of India and NFHS-III. Another limitation of this work is in counting the number of TB cases, that is those who are currently affected and those who suffered in the past are also combined together. Slum households classified by both commonly NFHS-III and Census were taken for the analysis, but for weight calculation Census data are used.

5. Conclusion

It is concluded that Chennai and Kolkata slums have approximately similar TB prevalence, but TB prevalence in Mumbai slum is ahead of these two cities. People classified under low SLI have higher risk for developing TB, and it is almost four times more than those who are categorised under medium or high SLI. Subsequently, place of dwelling is the risk factor; slum dweller has more risk of developing TB than those who are dwelling in non-slum areas. People who are dwelling in households without windows or electricity are approximately having the similar pattern of risk for developing TB than their counterparts. Hence, SLI, place of residence, windows and electricity in the households are the factors associated with TB prevalence.

Conflicts of interest

The author has none to declare.

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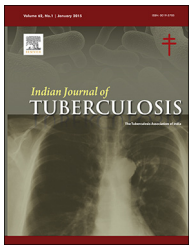

Original Article

Patterns of granulomatous responses in TB lymphadenitis and their correlation with treatment outcomes

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ABSTRACT

Introduction: Four patterns are noted in aspirates of TB lymphadenitis with or without concomitant HIV. They are granulomatous, necrotizing granulomatous, predominantly necrotizing and necrotizing suppurative designated pattern 1, 2, 3 and 4, respectively. The present study attempted to correlate granulomatous patterns, Acid Fast Bacilli (AFB) density with treatment outcomes.

Materials and methods: The MGG and Papanicolaou stained slides of 56 lymphadenitis patients, 38 TB and 18 TB with seropositive HIV were studied for two years. The AFB were stratified into: 0 – nil (1 – ≤1 AFB, 2 – >1 but <10 AFB, 3 – ≥10 AFB)/10 fields.

Results: There were 35 males and 21 females. Eleven aspirates demonstrated AFB. TB+HIV lymphadenitis displayed a higher AFB score. TB+HIV lymphadenitis aspirates significantly showed higher grade granulomas and AFB. TB+HIV lymphadenitis required ≥8-month treatment. Granulomas (pattern 3 or 4) but not high AFB scores required longer treatment (>6 months). Treatment of AFB (≥1) often extended to >6 months.

Conclusion: TB with seropositive HIV, possibly due to defective immune regulation exhibited granulomas (pattern 3 or 4) necessitating treatment for ≥8 months. Pattern 3 or 4 granulomas irrespective of HIV status demanded >6-month treatment.

1. Introduction

The cytomorphology of TB lymphadenitis in AIDS patients falls principally into either of four patterns: necrotizing, necrotizing suppurative, necrotizing granulomatous and granulomatous lymphadenitis.[1] Of these, necrotizing suppurative is the most confusing since any pointer to the TB infection is missing, this pattern being largely non-specific and can be seen in fungal infections, destructive metastases and other inflammatory conditions. The ill-formed granulomas of the necrotizing and necrotizing suppurative patterns are largely realized with very low CD4 counts.[2]

In a seminal article by Rao et al., these four patterns, albeit with a little difference were correlated with CD4 counts. The necrotizing and necrotizing suppurative with Acid Fast Bacilli (AFB) grade 3+ were clubbed into pattern 1. The granulomatous pattern was divided into pattern 4 without necrosis, AFB 1+ and pattern 3 with minimal necrosis and AFB 1+. The necrotizing and necrotizing suppurative pattern were

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associated with the lowest CD4 counts. The CD4 counts increased in quantum leaps as the granulomas became more defined and AFBs lessened. Thus, it was concluded that pattern 1 suffered from the most severe disease and pattern 4, the least.[3]

Even, in non-HIV patients with TB lymphadenitis, the gamut of the granulomatous responses is not too different from that of HIV positive cases, though necrotizing suppurative may be very occasionally encountered. The treatment outcome of these patients may then be correlated with the cytomorphological pattern. This would test the clinical implications of the patterns encountered in TB lymphadenitis and envisage the likelihood of therapeutic consequence should a particular pattern be faced, conditional upon significant concordance or the absence of such being established between the pattern and the treatment result. Such a study has so far not been undertaken in the literature.

2. Materials and methods

2.1. Study setting

The patients studied were recruited from the patients subjected to Fine Needle Aspiration Cytology (FNAC) of a lymph node. All the patients demonstrating granulomatous inflammation in their aspirates were checked for infection with *M. Tuberculosis*. The patients proven by culture positive *M. Tuberculosis* on their lymph node aspirates were taken up for this study. Since all the patients were included, the sampling method so used was opportunity sampling.

2.2. Method of data collection and duration of study

The archival FNAC slides were collected from the department of pathology which are already diagnosed with TB lymphadenitis. The patient's clinical data included the age and gender, duration of illness, blood counts and mycobacterial culture report. The treatment regimen prescribed by the physician was noted and record of periodic visits by the patient to document his/her disease status was also checked. *Retrospective study was continued for 2 years*. Completed records remained deficient in most cases and thus were not able to be incorporated in the study. Prior to two years, the slides were faded.

2.3. Laboratory methods, grading criteria and statistical methods

We collected and studied the Leishman/May-Grunwald-Giemsa (MGG) and Pap and Ziehl-Neelsen (ZN) stained slides already available in the FNAC laboratory. We grouped the granulomatous response into four patterns; the granulomatous and the necrotizing granulomatous effects occupying pattern 1 and 2 respectively; the necrotizing and necrotizing suppurative reactions occupying pattern 3 and 4 respectively. Pattern 1 granulomatous response should show well-formed granulomas composed of epithelioid cells, few Langhan's giant cells in a background of small lymphocytes or admixed with reactivated lymphoid cells with minimal caseous necrosis in the background. Pattern 2 granulomas likewise should show abundant caseous necrosis in the background with few epithelioid granulomas along with small lymphocytes. Pattern 3 granulomas should show no/occasional granulomas or scattered epithelioid cells with caseous necrosis largely ruling the aspirate picture. Pattern 2 and 3 should be differentiated by whether the granulomas can be found easily or have to be hunted for. Pattern 3 is ≤2 small granulomas in two well-spread slides with good material. Pattern 4 granulomas should not show granulomas at all, rather, very little necrotic material in the background with karyorrhectic debris, numerous neutrophils and scattered macrophages, not the epithelioid type should be the dominant aspirate representation. The number of AFB found were also stratified from numerous to absent into 4 grades; the absent scoring 0, 1 or less AFBs in 10 oil immersion fields (OI) scoring 1, more than 1 but less than 10 AFB per 10 OI awarded 2 and 10 or more AFBs in 10 OI notching a score of 3. The outcome of the treatment was correlated with the pattern of granulomas and the AFB score.

SPSS version 16 was used to analyze the data. Chi-square test was used to establish associations. A p-value of <0.05 was considered statistically significant.

3. Results and analysis

The duration for which slides could be studied spanned from December 2014 to January 2012. The total number of patients (n) were 56 of which 35 were males and 21, females.

Most patients (n = 23/56, 41.1%) belonged to the 20–29-year age group that consisted of 15 males and 8 females. The mean age of the patients was 33.23 ± 14.21 years, displaying a range starting at 10 up to 69 years.

Of all the patients, 38 suffered from TB only and 18, TB with HIV infection. The granulomas in majority of the cases (n = 15/18, 83.3%) of TB+HIV lymphadenitis showed pattern 3 or 4 granulomas. The TB lymphadenitis patients mostly displayed pattern 2 or 1 granulomas (n = 30/38, 78.9%) except 8 cases with pattern 3 or 4 granulomas. This difference in the granuloma patterns between TB and TB+HIV lymphadenitis patients was significant (Chi-square = 10.398, p = 0.0013). Of the 18 TB+HIV lymphadenitis cases, 5(27.78%) showed AFBs in their aspirate while 8(21%) of the TB lymphadenitis patients showed AFBs in their aspirate, the difference not being significant.

Most patients (n = 45/56, 80.4%) had an AFB grade of '0'. Of the 5 patients with AFB grades of 2 or 3, four belonged to the TB +HIV lymphadenitis and only one to TB lymphadenitis alone.

Most of the patients (n = 49/56, 87.5%) were prescribed Category I treatment. The mean duration of treatment was 6.98 ± 2.03 months. The duration of treatment taken by the patients ranged from 2 to 11 months.

The duration of treatment of TB lymphadenitis patients extended to less than 8 months, except 7 (18.42%) cases whereas most of the TB+HIV lymphadenitis patients (n = 12/18, 66.67%) were treated for 8 or more months. The difference in treatment duration between the two groups was significant (Chi-square = 12.69, p = 0.0004).

The granuloma pattern among all the patients was correlated with the final outcome vis-à-vis completed treatment versus discontinued/died and is shown in Table 1. The

Table 1 – The pattern of granuloma with the outcome of treatment (outcome 1 = completed treatment, outcome 2 = discontinued/died).

Pattern of granuloma	Patients with outcome 1	Patients with outcome 2	Total number of patients
Pattern 1 (number of patients)	11	1	12
Percentage	91.7%	8.3%	100.0%
Pattern 2 (number of patients)	20	1	21
Percentage	95.2%	4.8%	100.0%
Pattern 3 (number of patients)	12	1	13
Percentage	92.3%	7.7%	100.0%
Pattern 4 (number of patients)	10	0	10
Percentage	100.0%	.0%	100.0%
Total (number of patients)	53	3	56
Percentage	94.6%	5.4%	100.0%

Chi-square = 0.930, degrees of freedom = 3, $p = 0.818$, not significant.

pattern of granulomas did not have a bearing on the final outcome of 'completed treatment' as opposed to 'discontinued or dead'.

AFB is correlated with the outcome of treatment similar to that of pattern of granulomas. Like the granuloma patterns, AFB scores did not have any significant impact on the treatment outcome.

Table 2 shows the correlation between the pattern of granulomas of all the patients and the duration of treatment with regard to ≤6 months duration versus ≥6 months of total treatment. Pattern 2 or 1 granulomas were demonstrated in 33, pattern 3 or 4 were appreciated in 23 aspirates respectively. The duration of treatment significantly correlated with the pattern of granulomas, the patterns 3 or 4 generally claiming a longer period of treatment. Of the patients with longer duration of treatment ($n = 17$) and pattern 3 or 4 granulomas, 12 belonged to the TB+HIV lymphadenitis group while five suffered from TB lymphadenitis alone. Of the six aspirates with pattern 3 or 4 granulomas and treatment duration ≤6 months, three each belonged to the TB+HIV and the TB alone group respectively. Of the patients with pattern 1 or 2 granulomas, 20 needed treatment for ≤6 months while 13, ≥6 months.

Table 3 correlates AFB scores of all the patients with the total period of treatment. AFB scores did not correlate significantly with the total period of treatment. However, AFB scores of 1 or more required a treatment duration extending to more than 6 months in an overwhelming majority ($n = 8/11$, 72.7%).

The granulomatous patterns are demonstrated in Figs. 1 and 2. The granulomatous and necrotizing granulomatous patterns viz. patterns 1 and 2 are shown in Fig. 1. The necrotizing and necrotizing suppurative patterns viz. patterns 3 and 4 are shown in Fig. 2.

4. Discussion

In the present study, the 20–29-year age group had the maximum number of patients ($n = 23/56$, 41.1%), compatible with the review article statements of Gupta[4] and Mohapatra,[5] where a younger age group is favored in TB lymphadenitis.

TB lymphadenitis in patients with HIV generally displays one of the four patterns. Numerous well-formed granulomas, absent necrosis and 1+ AFB is labeled pattern 4. Pattern 3 shows well-formed granulomas, minimal necrosis and 1+ AFB. Pattern 2 does not display prominent granulomas, rather necrosis is predominant with 2+ AFB. Pattern 1 shows predominantly necrosis, may be suppurative with absent or occasional granulomas with 3+ AFB. All these patterns significantly associate with a distinct range of CD4 counts, pattern 1 with 54 ± 26.7, the lowest, pattern 2, 3, and 4 with 137 ± 24.4, 343 ± 41.9, 466 ± 26.8 CD4 cells respectively.[3] In our study, a completely reversed classification was used i.e.

Table 2 – The pattern of granulomas with the duration of treatment.

Pattern of granuloma	Duration		Total
	≤6 months	≥6 months	
Pattern 1 (number of patients)	10	2	12
Percentage	83.3%	16.7%	100.0%
Pattern 2 (number of patients)	10	11	21
Percentage	47.6%	52.4%	100.0%
Pattern 3 (number of patients)	5	8	13
Percentage	38.5%	61.5%	100.0%
Pattern 4 (number of patients)	1	9	10
Percentage	10.0%	90.0%	100.0%
Total (number of patients)	26	30	56
Percentage	46.4%	53.6%	100.0%

Chi-square = 12.25, degree of freedom = 3, $p = 0.007$, highly significant.

Table 3 – The correlation between AFB scores and the duration of treatment.

AFB score	Duration		Total
	≤6 months	≥6 months	
Score 0 (number of patients)	23	22	45
Percentage	51.1%	48.9%	100.0%
Score 1 (number of patients)	2	4	6
Percentage	33.3%	66.7%	100.0%
Score 2 (number of patients)	1	1	2
Percentage	50.0%	50.0%	100.0%
Score 3 (number of patients)	0	3	3
Percentage	0.0%	100.0%	100.0%
Total (number of patients)	26	30	56
Percentage	46.4%	53.6%	100.0%

Chi-square = 3.421, degrees of freedom = 3, $p = 0.331$, not significant.

pattern 1 in the study by Rao et al.[3] gets a designation of pattern 4 in our study.

In the present study, a modification to these patterns was used. Pattern 1 was split into predominantly caseous necrosis type or suppurative inflammation type which were patterns 3 and 4 to ensure better reproducibility like Nayak et al.[1] Pattern 3 and 4 were clubbed into grade 1. AFB was not considered here because very few cases demonstrated them. Also, these patterns were extrapolated to the non-HIV TB lymphadenitis cases as well.

In the study by Nayak et al., the pattern of granulomas among the TB lymphadenitis cases were 1 or 2 in the majority (15/21, 71.43%) while the TB+HIV lymphadenitis cases exhibited granulomas belonging mostly to patterns 3 or 4 (15/21, 71.43%)[1] as with Sridhar et al.[2] Echoing this observation, most TB+HIV lymphadenitis cases (15/18, 83.3%) in the present study allowed for pattern 3 or 4 granuloma while most of the non-HIV TB lymphadenitis cases ($n = 30/38$, 78.9%) exhibited a pattern 1 or 2 granuloma. The pattern 3 or 4 granulomas in HIV seropositive patients co-infected with *Mycobacterium*

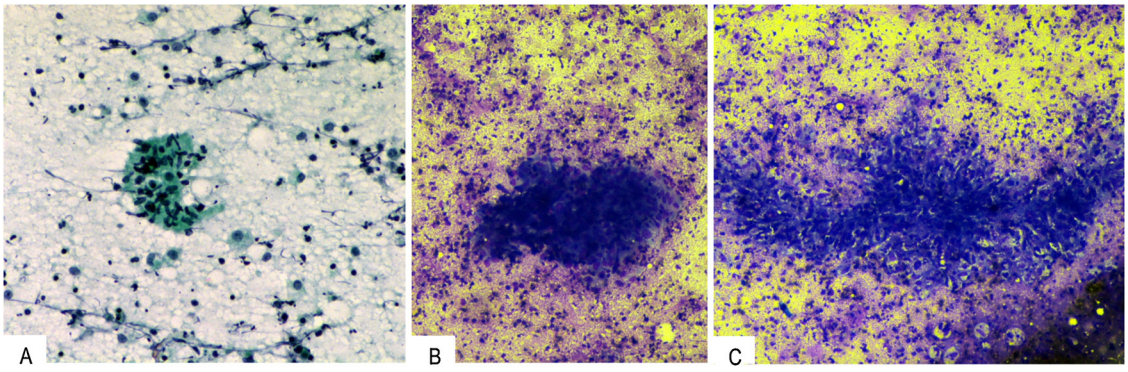

Fig. 1 – A – pattern 1, B&C – pattern 2. (A) Well-formed epithelioid granuloma in a background of streaked lymphocytes and RBCs (Pap X200). (B&C) Large epithelioid granulomas in a background of caseous necrosis and karyorrhectic debris (MGG X200).

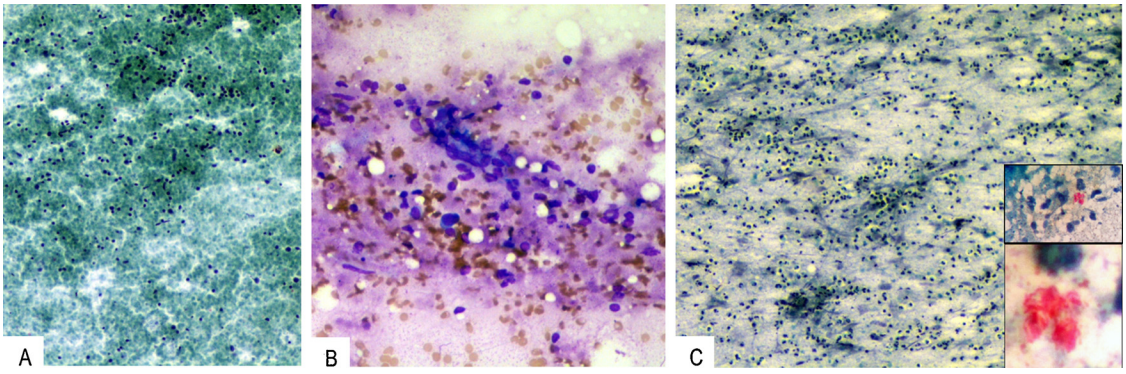

Fig. 2 – A&B – pattern 3, C – pattern 4. (A) Caseous necrotic debris with lymphocytes (Pap X100). (B) (same aspirate as A) Histiocytes, some with epithelioid morphology, loosely clustered in a background of casesous necrosis (MGG X200). (C) Necrotic debris with neutrophils, inset: AFB in clumps (HIV+ TB lymphadenopathy) (Pap X100, inset: ZN stain X400).

Tuberculosis is well anticipated response. Since the formation of well-formed granulomas require normal or adequate CD4 cells and the presence of CD25 on the T-cells and since their population dwindle in HIV seropositive patients, granulomas find it challenging to organize effectively. Also, migration of macrophages and their activation suffer a setback in HIV seropositive patients.[6]

Detection of AFBs remained low in the present study. The ZN stained smears had scant material generally. It is a common practice among the cytologists to assign the spreader slide to AFB staining owing to the mistaken perception that the spreader has touched all the slides. Thus, any slide with AFB will have lent some AFBs to the spreader. In practice, it has not worked. AFBs by and large, except in severely immunocompromised or heavily infected patients are very few to be found. Thus, in the spreader slide, finding AFB becomes coincidental rather than a fruit of careful examination. Though AFBs were found among both TB lymphadenitis and TB+HIV lymphadenitis cases, they were mostly found in cases with pattern 3 or 4 granulomas, though this observation was not statistically significant because of low number of cases.

The present study discovered that grade 4 granuloma particularly with 3+ AFB is significantly found in TB+HIV. Nayak et al.[1] and Rao et al.[3] too support this opinion. A suppurative necrotic pattern if found on the aspirates of HIV positive patients suspected of TB lymphadenitis should be confirmed with AFB positivity, a fact concluded by Shenoy et al.[7] also supported by the present study. All the suppurative necrotic smear pattern in TB+HIV lymphadenitis cases (6/18) in the present study were positive for AFB (5/6) except one which had inadequate material for AFB.

None of the cases in the present study suffered from pulmonary TB. Most of the patients were administered Category I or Category II treatment, though Category III treatment could have been granted to the HIV seronegative TB lymphadenitis cases.[8] The present study also attempted to correlate the pattern of granulomas with treatment response. This endeavor is unprecedented and convincingly highlights two substantial, statistically significant facts. TB with HIV lymphadenitis patients required a significantly longer duration of treatment than TB lymphadenitis alone since the expected positive response to treatment were not seen in most cases of HIV seropositive patients co-infected with TB. The implicit corollary that the absence of timely positive response to the TB medications may serve as a pointer to look for HIV co-infection may be true but needs a larger study to establish itself statistically. Again, another statistically significant observation in the present study was that smears belonging to patients with patterns designated a higher score demanded a longer duration of treatment that is more than 6 months with the conventional first line drugs, irrespective of whether they suffered from TB+HIV lymphadenitis or non-HIV TB lymphadenitis.

5. Conclusion

A significant correlation exists between the granulomatous response and the association of HIV seropositivity with TB. Pattern 3 (necrotic) or 4 (suppurative necrotic) granulomas were significantly more likely to be found in TB+HIV lymphadenitis patients than their TB only counterparts (Chi-square = 10.398, $p = 0.0013$). TB+HIV lymphadenitis patients also required a longer duration of treatment extending to 8 months or more (Chi-square = 12.69, $p = 0.0004$). If the aspirates showed a pattern 3 or 4 granuloma, irrespective of whether their patients suffered from TB alone or TB with concomitant HIV infection, they required a longer duration (>6 months) of treatment (Chi-square = 12.25, $p = 0.007$). Thus, it behoves the pathologist to assign a pattern to the granuloma in any case of TB lymphadenitis. A pattern 3 or 4, if encountered will alert the physician to prepare for a longer duration of treatment, if required than that recommended in Category I disease.

Author's contribution

Author 1 – concept and design of study, analysis and interpretation of data, drafting of the article, critical revision for important intellectual content and final approval of the submitted version.

Authors 2, 3 and 4 – acquisition, analysis and interpretation of data, revision of the article critically for important intellectual content and final approval of the submitted version.

Conflicts of interest

The authors have none to declare.

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Original Article

Prediction equations for spirometry in adults in western India

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ABSTRACT

Background: Spirometry is an essential investigation in pulmonology. The predicted normal spirometry values depend on various physiological parameters. This study was conducted to collect updated information on pulmonary functions in normal adults from western India.
Material and methods: A prospective observational study was undertaken at a tertiary hospital in Mumbai enrolling healthy subjects, 18–75 years, with ethnic origin from western India. Spirometry measurements were carried out as per ATS/ERS-2005 guidelines using a non-heated Fleish Pneumotachograph spirometer. Data was analyzed using SPSS for Pearson's correlation analysis, multiple linear regressions and log transformations of variables to get the best prediction equations.
Results: 310 subjects (185 males, 125 females) were included. Lung function values were higher in men as compared to women. In multivariate linear regression models, age and height were major predictor variables for all spirometry parameters. Addition of weight as a determinant variable did not make significant contribution to the models except for PEFR in males and F_{75} in females. Regression equations were established for FVC, FEV_1, FEV_1/FVC ratio, PEFR, $F_{25–75}$, F_{50}, and F_{75}. The standard-error-of-estimate was provided to enable computation of lower limits of normal for these parameters.
Conclusion: We propose regression equations for spirometry variables developed using the current standards for adult West Indian population fulfilling the long-felt need for updated equations.

1. Introduction

Spirometry is a vital investigation carried out by most pulmonologists. Interpretation of spirometry data classifies the severity of the underlying obstructive or restrictive abnormality. However, the interpretation of normal and disease depends on the predicted values. The predicted values depend mainly on anthropometry parameters, gender and ethnicity, though environmental, genetic, socioeconomic, and technical factors also contribute. Wide variations have been observed in diverse ethnic groups. Studies conducted in the

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different regions of India suggest the same diversification due to presence of various ethnic groups in our country.[1] Not only India in the US also NHANES and Strong heart studies[2] have pointed out the differences in various racial groups. Inappropriate choice of prediction equations may lead to errors in interpretation of spirometry data affecting management.[1] Therefore, it is imperative that locally valid equations be used in pulmonary function laboratories. Initial studies in Mumbai date back to 1970s.[3] However, no study has been conducted in west Indian population after 1987[4] to know the compatibility of predicted values. Technical and procedural aspects of spirometry have undergone major changes since then and the most recent statement of standardization was published by the task force of the American Thoracic Society and the European Respiratory Society (ATS-ERS) in 2005.[5] A significant cohort effect also exists such that the general health of a population undergoes changes due to changes in the environmental exposures. Thus, there is an urgent need to regularly update prediction equations in any population. No study has been carried out after the previously mentioned ones[3,4] in western India using the 2005 standardization of spirometry. In order to address this gap in information, we carried out a study to develop prediction equations for spirometry parameters for the western Indian population.

2. Material and methods

This was a prospective observational study carried out at a tertiary hospital in Mumbai as a part of the Indian Council of Medical Research (ICMR) study conducted from March 2009 to March 2012 at four centers nationwide after ethics approval. The sample size taken for the various centers was Mumbai 310 (185 males, 125 females), Delhi 685 (489 males, 196 females), Bangalore 407 (275 males, 132 females), and Kolkata 238 (92 males, 146 females). The data was analyzed at the Delhi center which was the coordinating center. Subjects aged 18 years and above were drawn after a written informed consent from a wide social and economic background, both urban and rural, from the eligible attendants of patients, healthy volunteers from Institutions, general public, private, and public sector offices. The minimum sample size recommended for multivariate regression analysis for lung function parameters is 150.[6] Age distribution matched the adult population of India according to the Census 2011 data.[7] After taking a written informed consent, detailed history and examination were done. A standardized respiratory questionnaire, based on the British Medical Research Council questionnaire was administered. A chest X-ray was performed. Smokers, subjects with past or current history of chronic respiratory diseases, thoracic cage abnormalities, cardiac or systemic diseases, recent upper or lower respiratory tract infection, and those not willing to give consent were excluded. Spirometry was done in all subjects. Spirometry was carried out according to the standardization recommendations of the American Thoracic Society/European Respiratory Society Task Force.[5] A Fleisch Pneumotach spirometer (KOKO, nSpire, UK) was used. The handle of the pneumotach contained the analog-to-digital converter and the digital signal was fed into the computer for analysis by nSpire software. The spirometer was calibrated daily according to the manufacturer's instructions using a 1 L syringe. The room temperature, barometric pressure, and humidity were noted and entered into the software. Then, details of the subject including date of birth, gender, height, and weight were entered. The maneuvers were performed in the sitting position with a nose-clip applied. The subject was asked to inhale completely and rapidly with a pause of <1 s at TLC and exhale with maximum force until no more air was expelled out while maintaining an upright posture. At the completion of expiration and on signal from the technician, the subject was asked to inhale completely. The maneuver was monitored on the computer screen. Throughout the procedure, loud verbal encouragement was given to obtain the expiratory and inspiratory maneuvers completely with maximal force. The technician observed the subject for distress, and also the computer display during the test to help ensure maximal effort with quality control as recommended by the ATS/ERS Task Force. The physician was always present to supervise. Quality control was ensured as described in the ATS-ERS statement.

Statistical analysis was done at the Delhi center. It was carried out using SPSS 20.0 (SPSS Inc., Chicago, USA) and Graph Pad Prism 4.01 (Graph Pad Inc., USA) software. Data from men and women was analyzed separately. Data was presented as mean $\pm$ SD, and as percentages as appropriate. Pearson's correlation analysis and univariate regression, both linear and nonlinear, were carried out to identify the significant predictor variables from among age, height, and weight for each of the dependent variables. Prediction equations were developed using the multiple linear regression procedure. Linear and nonlinear models were developed. Log transformations of dependent and other transformations of the independent variables were carried out to get the best model. Final models were selected considering simplicity and ease of clinical application, highest predictive capability (R^2), and satisfaction of assumptions of regression analysis. The goodness of fit was examined by testing for independence of predictor variables and the normality of the residuals. Unusual and influential observations were examined. These included outliers (standardized residuals more than ± 3), points with high leverage, and high influence. Analysis was repeated excluding these observations to determine their impact on the models and the original models were retained if the effect on the equations was small and inconsequential. Bland Altman analysis was carried out to evaluate the agreement of predicted and observed values to check for accuracy of the models. Internal validation of the derived models was done using the boot-strap resampling procedures, which were based on 2000 replications, size equal to original sample and with replacement separately for each gender. For each bootstrapped sample, new models were developed using the predictors present in the final model. The bias in regression coefficient (RC) of each predictor was calculated by the difference between average RC value from bootstrapped samples and RC value obtained from original sample. The previous equations in the same population[4] were applied to the study sample and data was compared with predicted values from the current equations using the unpaired ''t'' test.

Table 1 – Age distribution according to gender.

Age group	Males (n = 185)	Females (n = 125)
18–20 years	11 (5.9%)	16 (12.8%)
21–30 years	55 (29.7%)	23 (18.4%)
31–40 years	33 (17.8%)	20 (16.0%)
41–50 years	52 (28.1%)	36 (28.8%)
51–60 years	20 (10.8%)	17 (13.6%)
>60 years	14 (7.6%)	13 (10.4%)

Table 2 – Lung function (spirometry) data.

Parameter	Males (n = 185)	Females (n = 125)	p value
FVC (L)	3.68 ± 0.63	2.54 ± 0.50	$p < 0.0001$
FEV_1 (L)	3.03 ± 0.57	2.13 ± 0.47	$p < 0.0001$
PEFR (L/s)	8.30 ± 1.35	5.71 ± 1.02	$p < 0.0001$
F_{25-75} (L/s)	3.28 ± 1.02	2.52 ± 0.99	$p < 0.0001$
F_{50} (L/s)	4.04 ± 1.19	3.08 ± 1.13	$p < 0.0001$
F_{75} (L/s)	1.46 ± 0.65	1.21 ± 0.66	$p < 0.01$
FEV_1/FVC ratio)	82.23 ± 5.95	83.92 ± 7.62	$p < 0.05$

Significance observed with respect to gender.

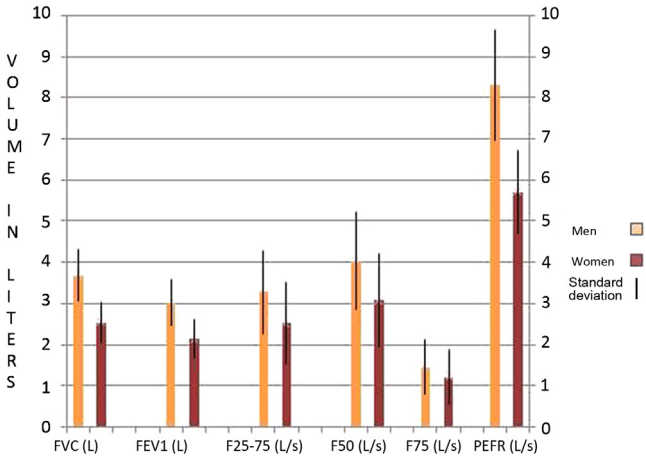

Fig. 1 – Graphical representation of lung function data for males and females.

3. Results

Total 310 subjects (185 [59.7%] males, 125 [40.3%] females) were included as they provided maneuvers that were technically acceptable and met the quality control. Age ranged from 18 to 82 years in men and 18 to 72 years in women. Table 1 displays the age distribution of the study population. The mean (±SD) age, weight, and height in men was 38.92 (±13.69) years, 65.07 (±10.41) kg, and 166.14 (±7.02) cm, respectively. The mean (±SD) age, weight, and height in women was 40.13 (±14.71) years, 54.97 (±11.09) kg, and 152.92 (±6.13) cm. The lung function parameters are presented in Fig. 1 and Table 2 suggesting significantly higher values in men in comparison with women. Forced vital capacity (FVC) and Forced expiratory volume in one second (FEV_1) correlated strongly with age and height in men and women. FEV_1/FVC showed a negative and stronger correlation with age in both genders. For FEV_1/FVC ratio, age was the only significant determinant in females and males. The explained variance ranged from 23 to 54% in females and 15 to 60% in males. FVC correlated with weight in both groups but FEV1 showed a correlation with weight in men and not in women. Table 3 illustrates the correlation coefficients for age, height, and weight for FVC, FEV_1, and FEV_1/FVC. Peak expiratory flow rate (PEFR), FEF_{25-75}, and FEF_{50} showed a decline with age and height in both groups but FEF_{75} declined with age and height only in men. PEFR correlated significantly with weight in both groups (men: $r = 0.28$, $p < 0.0001$, women: $r = 0.22$, $p < 0.05$). The regression equations developed for spirometry variables in men are summarized in Table 4 and for women in Table 5. The comparative predicted spirometry values from various studies in men and women

Table 3 – Pearson correlation coefficients (r^2) for FVC, FEV_1, FEV_1/FVC with age, height, and weight.

	Males			Females		
	FVC	FEV_1	FEV_1/FVC	FVC	FEV_1	FEV_1/FVC
Age	$r^2 = 0.23$	$r^2 = 0.35$	$r^2 = 0.15$	$r^2 = 0.18$	$r^2 = 0.34$	$r^2 = 0.28$
Height	$r^2 = 0.46$	$r^2 = 0.37$	$r^2 = 0.004$	$r^2 = 0.36$	$r^2 = 0.34$	$r^2 = 0.02$
Weight	$r^2 = 0.08$	$r^2 = 0.05$		$r^2 = 0.05$	$r^2 = 0.01$	

Table 4 – Regression equations for spirometry variables (Males).

Spirometry parameter	R-square	Adjusted R-square	Equation	SE
LnFVC	0.587	0.583	−1.048 + 0.015 × ht − 0.0045 × age	0.111
FEV_1	0.618	0.614	−3.275 + 0.043 × ht − 0.020 × age	0.346
PEFR	0.280	0.268	−1.867 + 0.057 × ht − 0.023 × age + 0.024 × wt	01.08
LnF2575	0.312	0.305	0.044 + 0.009 × ht − 0.0115 × age	0.270
LnF50	0.210	0.201	−0.033 + 0.010 × ht − 0.008 × age	0.275
LnF75	0.401	0.395	−0.246 + 0.0078 × ht − 0.020 × age	0.352
FEV_1/FVC ratio	0.212	0.208	89.09 − 0.179 × age	4.73

Ht in cm, age in years, Wt in kg. Parameters with "Ln" prefixed are natural log transformed. SE, standard error.

Table 5 – Regression equations for spirometry variables (females).

Spirometry parameter	R-square	Adjusted R-square	Equation	SE
LnFVC	0.663	0.654	$-1.616 + 0.015 \times ht + 0.014 \times age - 0.000219 \times age^2$	0.097
Ln FEV_1	0.681	0.673	$-1.552 + 0.015 \times ht + 0.0043 \times age - 0.000144 \times age^2$	0.115
PEFR	0.290	0.272	$-1.777 + 0.044 \times ht + 0.057 \times age - 0.000914 \times age^2$	0.739
LnF2575	0.420	0.411	$-0.270 + 0.012 \times ht - 0.017 \times age$	0.318
LnF50	0.346	0.329	$-0.299 + 0.012 \times ht - 0.013 \times age$	0.311
F75	0.521	0.504	$0.273 + 0.019 \times ht - 0.064 \times age - 0.0057 \times wt + 0.000448 \times age^2$	0.445
FEV1/FVC ratio	0.308	0.296	$104.35 - 0.085 \times age + 0.00650 \times age^2$	6.34

Ht in cm, age in years, wt in kg. Parameters with "Ln" prefixed are natural log transformed. SE, standard error.

Table 6 – Predicted spirometry values (standard deviation-SD) from various studies in men and women in comparison with percentage difference (%D).

Equation	Year	Sex	No. of patients	FVC (SD)	%D	FEV1 (SD)	%D
Indian studies							
Saleem (North)	2012	Men	1974	4.56(0.7)	8.9	4.04(0.6)	18.7
		Women	1106	2.97(0.5)	0.3	2.69(0.4)	10.6
Kamat (South)	1982	Men	739	3.70(0.6)	11.9	2.85(0.5)	16.1
		Women	508	2.60(0.4)	13.6	2.06(0.3)	16.1
Chatterjee (East)	1988	Men	104	3.97(0.4)	4.9	3.23(0.3)	3.6
		Women	230	2.42(0.3)	20.7	1.99(0.3)	19.5
Udwadia (West)	1987	Men	472	3.81(0.6)	9	2.84(0.5)	16.5
		Women	288	2.89(0.4)	3.1	2.16(0.4)	11.4
Current Study (West)	2013	Men	185	4.17(0.4)	Ref	3.35(0.4)	Ref
		Women	125	2.98(0.4)	Ref	2.42(0.3)	Ref
Caucasian studies							
Quanjer	1993	Men	189	4.57(0.6)	9.2	3.93(0.4)	15.9
		Women	514	3.25(0.4)	8.7	2.80(0.4)	14.6
Knudson	1983	Men	86	4.64(0.6)	10.7	3.81(0.5)	12.8
		Women	204	3.36(0.5)	12	2.79(0.4)	14.2
Capro	1981	Men	125	4.89(0.6)	15.9	3.96(0.5)	16.7
		Women	126	3.54(0.4)	17.2	2.92(0.3)	18.7

The predicted values are calculated for 45-year-old male, 175 cm height and 45-year-old female, 165 cm height for ease of calculation and comparison. Ref, reference value.

(45 years male 175 cm height, 45 years female 165 cm height) are represented in Table 6. Comparison of predicted values derived from the study equations and Indian reference standards (IRS) was done in a subset of independent patient cohort as illustrated in Fig. 2. The comparative scatter grams for FVC and FEV_1 for study values in comparison with the IRS values are depicted in Fig. 3.

4. Discussion

Spirometry studies have been carried out in western India since 1960s.[3,4] The major study was done by Udwadia et al.[4] way back in 1987 at Mumbai following which Mohan Rao et al.[8] studied healthy subjects in 1992 at Ahmedabad, Gujarat. After all these years, we report this latest study from West India. The current study findings suggest the correlation of FVC and FEV_1 with studies done in Mumbai (Udwadia et al.[4]), however, with higher values in women as compared with South India (Kamat et al.[9]). The FVC and FEV_1 values observed in men were lower when compared to the studies done in Northern India (Jain et al.[10] and Saleem et al.[11]). However, in our study, women had comparable FVC and FEV_1 as in the North. FVC and FEV_1 were lower in our study in comparison with other international studies.[12–15] Lung function variation has been a known phenomenon noted in various studies done in India and West. The comparative Table 6 is illustrated.

Western India equations were first and latest proposed by Udwadia et al.[4] Mumbai being a cosmopolitan city, as against the study by Udwadia et al.,[4] we included the subjects from Mumbai of ethnic origin from western India only. As in Udwadia's study, our study observed significant correlation of FVC and FEV_1 with age and height and of FEV_1/FVC ratio with age. The FEV_1/FVC ratio in both men and women declined with age as was demonstrated by Udwadia et al. In contrast with the study by Udwadia et al., we observed that FVC and FEV_1 of all age groups in both males and females demonstrated a strong negative correlation with age suggesting decline with increasing age. Hence a standardized equation was formulated applicable for all adults as against separate equations for those below and above 30 years of age in the Udwadia study.[4] The predicted equations of our study in

STUDY = PREDICTED DERIVED FROM OUR EQUATIONS PRED= PREDICTED DERIVED FROM INDIAN STANDARDS

Sr. No	Sex / Age / Ht(cm)/Wt(kg)		STUDY	PRED	Sr. No	Sex / Age / Ht(cm)/Wt(kg)		STUDY	PRED
			AGE GROUP LESS THAN 20 YRS						
1	M	FVC	4.37	4.46	2	M	FVC	3.91	4.05
	19	FEV1	3.72	3.8		20	FEV1	3.36	3.49
	173/63	PEFR	9.1	8.74		165/49	PEFR	8.35	8.3
			AGE GROUP 20-30 YRS						
3	F	FVC	3.38	3.55	4	F	FVC	2.84	3.03
	25	FEV1	2.93	3.11		23	FEV1	2.5	2.65
	169/80	PEFR	6.91	6.7		156/45	PEFR	6.1	6.06
5	M	FVC	4.64	4.79	6	M	FVC	3.49	3.74
	29	FEV1	3.85	4.01		25	FEV1	3.01	3.26
	181/77	PEFR	9.57	9.03		159/51	PEFR	7.96	7.97
			AGE GROUP 30-40 YRS						
7	F	FVC	2.99	3.06	8	F	FVC	2.58	2.71
	34	FEV1	2.55	2.66		32	FEV1	2.22	2.34
	162/62	PEFR	6.33	6.11		152/42	PEFR	5.72	5.67
9	M	FVC	4.08	4.21	10	M	FVC	4.26	4.37
	36	FEV1	3.38	3.51		38	FEV1	3.5	3.62
	173/87	PEFR	9.23	8.32		177/51	PEFR	8.5	8.46
			AGE GROUP 40-50 YRS						
11	F	FVC	2.41	2.44	12	F	FVC	2.35	2.36
	40	FEV1	2.03	2.1		42	FEV1	1.96	2.02
	150/62	PEFR	5.48	5.36		149/61	PEFR	5.39	5.25
13	M	FVC	3.04	3.19	14	M	FVC	3.5	3.61
	42	FEV1	2.54	2.7		44	FEV1	2.88	3
	156/62	PEFR	7.64	7.15		165/54	PEFR	7.85	7.56
			AGE GROUP 50-60 YRS						
15	F	FVC	2.35	2.25	16	M	FVC	2.65	2.75
	50	FEV1	1.92	1.91		52	FEV1	2.17	2.29
	151/53	PEFR	5.4	5.13		152/49	PEFR	6.95	6.53
17	M	FVC	3.44	3.48	18	M	FVC	3.66	3.69
	54	FEV1	2.76	2.81		54	FEV1	2.93	2.97
	167/80	PEFR	8.3	7.29		171/75	PEFR	8.53	7.51
19	F	FVC	2.03	1.8	20	F	FVC	1.9	1.73
	60	FEV1	1.58	1.5		57	FEV1	1.48	1.42
	146/65	PEFR	4.94	5.22		142/34	PEFR	4.73	4.5
			AGE GROUP 60-70 YRS						
21	M	FVC	3.29	3.27	22	M	FVC	2.88	2.89
	63	FEV1	2.58	2.57		64	FEV1	2.27	2.27
	167/63	PEFR	7.65	6.94		160/57	PEFR	7.14	6.5
23	F	FVC	1.96	1.71	24	F	FVC	2.02	1.77
	63	FEV1	1.5	1.4		62	FEV1	1.55	1.46
	145/67	PEFR	4.84	4.47		146/50	PEFR	4.91	4.55
			AGE GROUP MORE THAN 70YRS						
25	M	FVC	2.76	2.68	26	F	FVC	2.25	1.7
	75	FEV1	2.08	2.03		82	FEV1	1.62	1.36
	161/59	PEFR	6.95	6.13		156/51	PEFR	5.27	4.51

Fig. 2 – Comparison of predicted values derived from the study equations and reference Indian standards in a subset of patients.

Table 7 – Comparison of current study and Udwadia et al. study.

Study	Mean FVC ± SD men	Mean FEV_1 ± SD men	Mean FVC ± SD women	Mean FEV_1 ± SD women
Udwadia et al. (subjects >30 years)	3.296 ± 0.601	2.603 ± 0.452	2.394 ± 0.444	1.923 ± 0.389
Current	3.68 ± 0.63***	3.03 ± 0.57***	2.54 ± 0.50**	2.13 ± 0.47***

*** $p < 0.0001$.
** $p < 0.01$.

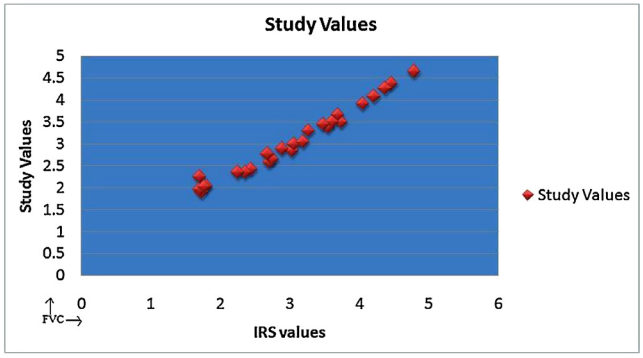

Fig. 3 – Scatter-grams comparing predicted values of FVC and FEV_1 derived from the study equations and reference Indian standards in a subset of patients.

comparison with previous West India study by Udwadia et al. were as follows:

1) For males

Study predicted FVC

$LnFVC = -1.048 + (-0.0045 \times Age) + (0.015 \times Height)$ (SEE = 0.11, $R^2 = 0.583$)

Udwadia et al. Predicted FVC(subjects < 30 years) $= -6.058 + (0.019 \times Age) + (0.055 \times Height)$ (SEE = 0.664, $R^2 = 0.422$)

Udwadia et al. Predicted FVC(subjects ≥ 30 years) $= -4.832 + (-0.018 \times Age) + (0.054 \times Height)$ (SEE = 0.601, $R^2 = 0.409$)

Study predicted FEV1

$FEV_1 = -3.275 + (-0.020 \times Age) + (0.043 \times Height)$ (SEE = 0.346, $R^2 = 0.614$)

Udwadia et al. Predicted FEV_1 (subjects < 30 years) $= -3.266 + (-0.010 \times Age) + (0.039 \times Height)$ (SEE = 0.495, $R^2 = 0.371$)

Udwadia et al Predicted FEV_1 (subjects ≥ 30 years) $= -2.650 + (-0.022 \times Age) + (0.037 \times Height)$ (SEE = 0.452, $R^2 = 0.473$)

2) For females

Study predicted FVC

$LnFVC = -1.616 + (0.014 \times Age) + (-0.000219 \times Age^2 + (0.015 \times Height)$ (SEE = 0.097, $R^2 = 0.654$)

Udwadia et al. Predicted FVC(subjects < 30 years) $= -2.284 + (0.006 \times Age) + (0.030 \times Height)$ (SEE = 0.418, $R^2 = 0.187$)

Udwadia et al. Predicted FVC(subjects ≥ 30 years) $= -3.755 + (-0.010 \times Age) + (0.043 \times Height)$ (SEE = 0.444, $R^2 = 0.412$)

Study predicted FEV_1

$LnFEV_1 = -1.552 + (0.0043 \times Age) + (-0.000144 \times Age^2) + (0.015 \times Height)$ (SEE = 0.115, $R^2 = 0.673$)

Udwadia et al. Predicted FEV^1 (subjects < 30years) $= -1.424 + (-0.011 \times Age) + (0.025 \times Height)$ (SEE = 0.373, $R^2 = 0.166$)

Udwadia et al. Predicted FEV_1(subjects ≥ 30 years) $= -2.580 + (-0.012 \times Age) + (0.032 \times Height)$ (SEE = 0.389, $R^2 = 0.367$)

One of the reasons for this variation could be the differences in distribution of population in the two studies. Udwadia et al. had 309 subjects who were less than 30 years of age as against 105 in our study and 451 who were 30 years of age or older as against 205 in our study. We found a statistically significant increase in the mean FVC and FEV_1 in both men and women as compared to the study done by Udwadia et al. The comparative data of both studies is mentioned in Table 7. This increase may be related to technical factors and due to a significant cohort effect wherein the general health of a population undergoes changes due to changes in the environmental exposures.

As universally known, lung function values were significantly higher in men as compared to women. However, our study highlights the possible correlation of weight as a variable parameter for FVC, FEV_1, and PEFR. A positive correlation of weight and FVC in both men and women was observed in our study. FEV_1 correlated with weight in men only. PEFR correlated strongly with weight in both groups as observed in a study from Odisha.[16] While the impact of bone free lean body mass has already been studied as an independent variable in predicting lung functions,[17] our study further reiterates the fact that correlation of body weight and its components with lung functions should be studied in larger populations. The comparative assessment of the predicted values derived from the study equations and existing IRS equations (done in an independent patient cohort) revealed agreement and good performance of the equations.

Our study had limitations of sampling strategy and sample size. Random sampling from within the whole population is ideal but difficult; hence, the alternative acceptable method of sampling healthy subjects was used in our study. For development of regression equations, a minimum sample size of 150 men and 150 women has been recommended. We had 185 men and 125 women subjects. Other limitations included inability to get healthy elderly subjects (>70 years) to perform acceptable spirometry. Hence, extrapolation of these equations in this group should be done with caution.

5. Conclusion

After a gap of two decades we propose updated regression equations for spirometric variables for the adult population of Mumbai above 18 years of age using current ATS/ERS 2005 standardization guidelines for lung function testing. Hence fulfilling the long-felt need for updating these equations, this will eventually prove useful in management of patients with respiratory diseases. Mumbai being a cosmopolitan city, ours is the first study wherein only subjects of ethnic origin from western India were studied exclusively. Various factors interplay a role in the vast variations of lung functions. This has been documented for age, sex, height in multiple studies for all populations. However our study suggests significant correlation of the weight parameter with FVC, FEV_1, and PEFR requiring larger population studies to confirm the hypothesis.

Conflict of interest

The authors have none to declare.

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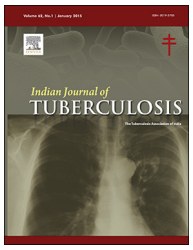

Original Article

Tobacco use and its impact on pulmonary health among elderly population in rural area of Muzaffarnagar – A cross-sectional study

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ABSTRACT

Background: The tobacco use is significant in Indian rural population. Among them, elderly people in rural area are at special risk due to ageing and other factors. The impact of tobacco use on elderly health, therefore, needs to be studied in depth in rural context.
Objective: To study the patterns of tobacco use and its consequent impact on pulmonary health of the elderly.
Design and methodology: A community-based cross-sectional study was done (April 1st to September 30th, 2014) in the field practice area (village Bilaspur) of Rural Health Training Centre (RHTC) of Muzaffarnagar Medical College, Muzaffarnagar. A simple random sampling was used and elderly of 60 years and above were interviewed by semi-structured interview schedule. The data were analyzed by software Epi-info. version 7.1.3.3.
Results and conclusion: The prevalence of tobacco usage among elderly was 56.7%, in which smoking was the dominant one (37%) and majority being in the form of Bidi (56.7%). Tobacco usage was significantly associated not only with age, sex, and caste ($p < 0.05$ each), but occupational and socio-economic status ($p < 0.01$ each) also; however, literacy was the most significant factor ($p < 0.0001$) among all. The tobacco usage in smoking form was highly significantly associated with the presence of chronic obstructive pulmonary disease ($p < 0.0001$), elucidating a significant impact on their pulmonary health. The rural elderly people need health education regarding curtailing the use of tobacco for their better health from health clinics.

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1. Introduction

Globally, tobacco accounts for at least 10% adult deaths and 6 million illnesses per year. Tobacco use is a serious public health problem in India, as between ages 30 and 69 years, it is causing 5% death among women and 20% death among men in India.[1] By 2020, it is expected that tobacco will be responsible for 13% deaths in India.[1–3] The cost of treatment of tobacco-related diseases and the loss of productivity in India is around Rs. 13,500 crores annually, which is more than the benefits gained in the form of revenue and employment generated by tobacco industry.[4]

India is the third largest producer and consumer of tobacco in the world, where cigarette consumption has reached to a level in causing serious tobacco usage related problems in the areas of both health and productivity.[5,6] According to the most recent figures from the Global Burden of Disease (GBD) study, coordinated by Institute of Health Metrics Evaluation (IHME), tobacco usage has led to nearly one million deaths and significant health loss in India.[7] Now, more Indian people are smoking (110 million, compared to just 74.5 million smokers three decades back), notwithstanding the presence of anti-tobacco and smoke-free laws.[7] According to the World Health Organization (WHO) figures, India has 12% of world's smokers and there are approximately 120 million smokers in India.[8] According to an old WHO estimate, 30% of adult males in India smoke more as compared to adult females (3–5%).[9]

Although the overall prevalence of tobacco use globally as per one systematic review with meta-analysis study was low in terms of 13% in both genders (22% males and 8% females), this must be considered very cautiously in the context of India.[10] According to a recent WHO report on Global Tobacco Epidemic (2013), currently more than 35% Indians are consuming tobacco, with an estimate of 59.6% smokeless tobacco and 25% smoked tobacco. It has also been found that the prevalence of tobacco consumption can not only increase up to the age of 50 years, but also the prevalence of smoking and chewing varies widely between different states of India with a strong association with individual's socio-cultural characteristics.[11] Socio-demographically, in India, tobacco smoking is more prevalent in men and among older people. Although men have been found to smoke throughout their lives, the women are also becoming smokers at an older age.[12–14]

Tobacco smoking, thus, is not only related to ill health, but also to an impaired functional capability in bone mineral density, pulmonary function, and muscle strength.[15] Chronic obstructive pulmonary disease (COPD) in elderly are now found to be significantly associated with tobacco usage in India. It has been found that the prevalence of COPD in the elderly with negative histories of smoking is low and this emphasizes the importance of reducing smoking as the effective preventive measure for elderly health.[16] Thus, there are many studies on adolescents and young adults on tobacco usage patterns in India, but the studies specifically researching the area of using tobacco and its consequent impact on lung health among elderly in rural area in district Muzaffarnagar of State UP, India (where socio-cultural and behavioral factors among elderly may be dominant due to its population dynamics) are lacking in literature. This was the prime reason for choosing this research area for study by authors as a part of research project funded by TB Association of India.

2. Methods

Ethical approval: This study was carried out under a research project approved both by TB association of India and Institutional ethics committee of Muzaffarnagar Medical College, Muzaffarnagar (UP).

Study design: A community-based cross-sectional study.

Study subjects: Elderly above 60 years of age.

Study duration: April 1st, 2014 to September 30th, 2014.

Study area: The study was conducted in the village Bilaspur in the field practice area of Rural Health Training Centre (RHTC) of Department of Community Medicine Muzaffarnagar Medical College, Muzaffarnagar (UP).

Sampling technique: The RHTC catering area covered a total population of 43,117 (up to September 30th, 2014). A total of six villages were first enlisted from this RHTC catering area, i.e., Shernagar, Makhiyali, Bilaspur, Sikheda, Dhandhera, and Bhagwanpuri. A simple random sampling was used to select a village by a lottery method. So the village Bilaspur, which got randomly selected, was considered for this study. The elderly who had been residents of this village Bilaspur for at least last 6 months and who were above 60 years of age were enrolled. A computerized list of the elderly was made of the study area and study participants were contacted by health workers in this area. From this list, sampled elderly were studied. The consent of elderly was also taken after explaining the importance of this study.

Methodology: All the study subjects residing in the field practice area in the village Bilaspur of Rural Health Training Centre (RHTC) of Department of Community Medicine Muzaffarnagar Medical College, Muzaffarnagar (UP) were enrolled and they were selected by using simple random sampling. Due to no clear-cut availability of prevalence among elderly in our study setting on tobacco usage, 50% prevalence was assumed as per WHO criteria and this also nearly corresponded with approximately at least 5% population of elderly out of total population of 8142 catered by Bilaspur village in RHTC catering area in the year 2014.

Sample size calculation:

$N = 4PQ/L^2$

$P = 50\%$ (assumed prevalence as per WHO criteria)

$Q = 50\%$ (100-P)

L = allowable error (10% of P) = 5

So, $N = 400$

A total of 400 elderly subjects were selected for this study, thereby completing the sample size.

Inclusion criteria: The criteria for selection of cases were as per working definitions of tobacco usage and COPD. Tobacco products were considered in our study comprising entirely or partly of leaf tobacco as raw material, which can be smoked, sucked, or chewed in the last 6 months. Tobacco use in our study was defined as any habitual use of the tobacco plant leaf

and its products as per the responses of subjects. A COPD diagnosis as per WHO guidelines was considered in our study – in any patient who had symptoms of cough, sputum production, or dyspnoea – for the disease. These were the main criteria considered for calculating the prevalence and studying the impact of tobacco use in elderly.

Exclusion criteria: However, dose response effect of tobacco usage on elderly health was excluded from this study.

Data collection technique: PG students, Medical interns, & field investigators were adequately trained for the purpose of the collection of data using a semi-structured interview schedule (questionnaire). First, a pilot study on 50 subjects was undertaken, and required correction or suggestion was incorporated accordingly in the questionnaire.

2.1. *Statistical analysis*

The data were tabulated into Epi-info. version 7.1.3.3 and analyzed by using this software. Nominal data were analyzed by using Chi-Square test for knowing statistical associations.

3. Results

3.1. *Prevalence of tobacco use as per socio-demographic status*

The overall prevalence of tobacco use was 56.7%, and this was more in the age group 65–70 years (63.7%), males (60.4%),

Table 1 – Prevalence of tobacco use under different Socio-demographic parameters of subjects (N = 400).[a]

	Tobacco-users		Tobacco Non-users		Prevalence	
	No.	%	No.	%	No	%
1. Age groups[b]						
60–65	113	49.7	74	42.7	187	60.4
65–70	65	28.7	37	21.3	102	63.7
70–75	37	16.3	36	20.9	73	50.7
>75	12	5.3	26	15.1	38	31.6
Total	**227**	**56.75**	**173**	**43.25**	**400**	**56.7**
2. **Sex wise**[c]						
Males	168	74.0	109	63.1	277	60.4
Females	59	26.0	64	36.9	123	48
Total	**227**	**100**	**173**	**100**	**400**	**56.7**
3. Religion wise[d]						
Hindu	66	29.1	44	25.4	110	60.0
Muslims	161	70.9	129	74.6	290	56.5
Total	**227**	**100**	**173**	**100**	**400**	**56.7**
4. Caste groups[e]						
SC & ST	53	23.3	23	13.2	76	69.7
OBC	55	24.2	35	20.2	90	61.1
General	119	52.4	115	66.4	234	50.8
Total	**227**	**100**	**173**	**100**	**400**	**56.7**
5. Literacy wise[f]						
Literate	93	40.9	117	67.7	210	44.3
Illiterate	134	59.1	56	32.3	190	70.5
Total	**227**	**100**	**173**	**100**	**400**	**56.7**
6. Occupation wise[g]						
Agriculture	146	64.3	85	49.1	231	63.2
Non-agriculture	81	35.7	88	50.9	169	47.9
Total	**227**	**100**	**173**	**100**	**400**	**56.7**
7. Socio-economic status wise[h]						
Class-I	16	7.1	13	7.5	29	55.1
Class-II	24	10.6	15	8.7	39	61.5
Class-III	115	50.6	71	41.1	186	61.8
Class-IV	56	24.7	41	23.6	97	57.7
Class-V	16	7.1	33	19.1	49	32.6
Total	**227**	**100**	**173**	**100**	**400**	**56.7**

[a] Prevalence calculated as per row wise percentage.
[b] Chi-Square test: $X^2 = 13.96$, d.f. = 3, $p < 0.05$.
[c] Chi-Square test: $X^2 = 5.57$, d.f. = 1, $p < 0.05$.
[d] Chi-Square test: $X^2 = 0.65$, d.f. = 1, $p > 0.05$.
[e] Chi-Square test: $X^2 = 9.23$, d.f. = 2, $p < 0.05$.
[f] Chi-Square test: $X^2 = 27.98$, d.f. = 1, $p < 0.0001$.
[g] Chi-Square test: $X^2 = 9.28$, d.f. = 1, $p < 0.01$.
[h] Chi-Square test: $X^2 = 13.98$, d.f. = 4, $p < 0.01$.

Hindus (60%) in SC–ST category (69.7%), and in illiterates (70.5%) with predominance in agricultural occupation (63.2%) and socio-economic class III (61.8%) (Modified BG Prasad classification) (Table 1).

3.2. Distribution of modes of tobacco use

The overall prevalence of tobacco smoking was 37% and smokeless tobacco was 19.7%. In the modes of tobacco usage, smoking was the dominant one (65.2%) in the form of Bidis (56.7%), whereas in smokeless form, Khaini was the main mode of usage (77.3%) (Tables 2 and 3).

Further, it was also observed that a total of 17 (7.5%) study subjects were using both forms of tobacco, i.e., exposed to both smoking and tobacco chewing. Assuming that smoking is more prone in causing pulmonary illnesses, all such subjects were allocated in the smoking category.

3.3. Prevalence of COPD among tobacco users and non-users

The prevalence of COPD among elderly was 22.2%, and among them, the prevalence of tobacco usage was 30.4%, that too mainly in the form of smoking (prevalence – 42.6%) as compared to smokeless form (prevalence – 7.6%) (Table 4).

4. Discussion

In the present study, the overall prevalence of tobacco use among elderly was 56.7%, and this was more in the age group of 65–70 years, with male predominance and among Hindus – that too in SC–ST category. Studies in the past[8] also indicate that nearly more than 50 percent of the tobacco-related morbidity and mortality in India occur among illiterates, and 80 percent of these people reside in rural India, which is similar to the findings in our study. The skewing of tobacco usage among elderly in illiterates with a predominant agricultural occupation and socio-economic class III (Modified BG Prasad classification – a socio-economic status scale capable of measuring socio-economic status of both rural and urban community based on per capita monthly income of the family)[17] indicates the possibility of influence of socio-economic, demographic, and cultural factors in tobacco usage among elderly in district Muzaffarnagar (UP),[18–20] which is similar to the patterns found in other studies. The higher prevalence obtained in our study (56.7%) is almost consistent with WHO (2009–2010) data[18–20] of figure in the range of 61% (between 45 and 65 years) and 55% in greater than 65 years age group. It was also consistent with the study by Goswami et al. (2005)[21] on elderly using tobacco, in which smoking tobacco ranged from 77% in the 60–64 years age group to 63.9% in the >75 years age in men ($p < 0.001$).

Moreover, tobacco usage in our study was not only significantly associated with age, sex, and caste of elderly ($p < 0.05$ each), but also more with the occupational and socio-economic status of elderly ($p < 0.01$ each). However, the most significant factor of literacy among elderly ($p < 0.0001$), which emerged from our study, can explain higher usage tobacco in rural area of Muzaffarnagar with a predominantly illiterate population with little knowledge of harmful effects of tobacco, which is similar to previous many surveys and reports of Government of India and WHO.[18–23] The report of Global Tobacco Epidemic (2013) also indicates on two significant factors to drive tobacco use in India: Education level and socio-economic status and Illiterate and poorer individuals tend to use more tobacco than those with more education or of higher economic status,[18,22] which is in line with our present study. According to India's National Family Health Survey also, Indians with no education were 2.69 times more likely to

Table 2 – Prevalence of different modes of tobacco use (N = 400).

Prevalence of modes of tobacco use	No.	%
Smoking	148	37.0
Oral form	79	19.7
Total	**227**	**56.7**
Non-users	173	43.3
Grand total	**400**	**100**

Table 3 – Distribution of predominant modes of tobacco product use (N = 227).

Types of tobacco use	No.	%
Smoked tobacco product use (n = 148)		
1. Cigarette	16	10.8
2. Bidi	84	56.7
3. Hukka	48	32.5
Total	**148**	**100**
Smokeless tobacco product use (n = 79)		
4. Gutkha	18	22.7
5. Khaini	61	77.3
Total	**79**	**100**

Table 4 – Distribution of subjects by tobacco usage status and Prevalence of COPD (N = 400).

Tobacco usage status		COPD status			Prevalence percentage of COPD
		Presence	Absence	Total	
Tobacco users (n = 227)	Smoked users (n = 148)	63	85	148	42.6
	Smokeless users (n = 79)	6	73	79	7.6
	Total	**69**	**158**	**227**	**30.4**
Tobacco non-users (n = 173)		20	153	173	11.6
Grand total (N)		**89**	**311**	**400**	**22.2**

Chi-square test: $X^2 = 56.5$, d.f. = 2, $p < 0.0001$.

smoke and/or chew tobacco than those with a postgraduate education.[22] Households categorized as being in the lowest fifth in the standard of living index were 2.54 times more likely to use tobacco than those in the highest fifth.[23]

In further analysis on modes of tobacco usage in our study, out of tobacco usage, smoking was the dominant one (65.2%) and this finding is in contrast with the report of Global Adult Tobacco Survey (GATS – 2009 & 2010) & report of Global Tobacco Epidemic (2013) by WHO, where they had reported that 26.6% of people used tobacco and more than 2 in 5 adult males and 1 in 5 adult females used tobacco.[18–20] When the type of smoking was further analyzed, it appeared that the overall prevalence of tobacco smoking was 37%, whereas smokeless tobacco was 19.7%, and this was also similar with the tobacco usage status report in the GATS-India fact sheet (2009–2010) of Khaini (18%).[20] The higher prevalence of smoking in our study (37%) as compared to 16% GATS-India fact sheet (2009–2010) may be due to easy availability of tobacco smoking products as well as other unknown socio-behavioral factors of elderly in study area. Moreover, in our study, smoked tobacco usage among elderly was mainly in the form of Bidis (56.7%), whereas in smokeless form, Khaini was the main mode of usage (77.3%) and this finding was also in unison to the report by Global Adult Tobacco Survey (GATS – 2009 and 2010),[18–20] which had found the highest number of Bidi smokers in few states such as Uttarakhand.

In our study, the prevalence of COPD among elderly was 22.2%, and among them, the prevalence of tobacco usage was 30.4%, that too mainly in the form of smoking (prevalence – 42.6%) as compared to smokeless form (prevalence – 7.6%), and this was also found to be statistically significant ($p < 0.0001$). The higher prevalence of COPD (22.2%) found in our study is in contrast to the study by Parasuramalu et al. (2014),[24] which found the prevalence rate of 4.36% and this can be explained due to some inherent socio-cultural factors found in our study area in which dominant smoking form in higher form (37%), which may be exposing lungs of elderly to a greater extent, associating with COPD in a firm way.

In our study, further the presence of COPD was highly significantly associated not only with the usage of tobacco (30.4% as compared to 11.6%) ($p < 0.001$) but also with the tobacco usage in the form of smoking as compared to smokeless tobacco users (52.7% as compared to 13.9%). This finding is similar to the study,[24] which has shown an increased occurrence of chronic respiratory symptoms and deficits in ventilatory lung function in relation to tobacco exposure at home and/or at work. This finding is also similar to the studies,[25,26] which have also found significant relations between tobacco exposure and COPD in the elderly. In general, tobacco smoking in any form is found to be a major risk factor, resulting in COPD that is in line to our study's finding.

Thus, in India, tobacco can act as a double edge sword for elderly health in rural areas, for which tobacco cessation interventions need to be directed towards elderly with lower literacy and poor socio-economic status, as also advocated in the study by Mini et al. (2014).[27] In our study, even Bidi smoking is not found to be less hazardous than cigarette smoking in elderly and smokeless tobacco use also appears to cause higher morbidity as found in other study,[28] so all forms of tobacco usage must be discouraged among elderly. Failure to contain tobacco use among elderly in India, therefore, can result in doubling the burden of non-communicable diseases such as COPD and hypertension, for which elderly health clinics imparting health education on curtailing tobacco use is the need of hour. Tobacco education can be imparted through existing government health programs and hospital outreach programs[29] and even by health training centers of Private Medical Colleges in India.

5. Limitations of study

In the present study for assessing impact of tobacco on lung health, 2 factors are the limitations: first, the dose response effect of tobacco on lung health was not seen and second, other diseases of lung including cancer, tuberculosis, etc., which were not considered, might influence the picture of complete impact of tobacco usage on pulmonary health of elderly.

Conflicts of interest

The authors have none to declare.

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Authors' contributions

SD, SKR, KM and RS have contributed equally to the article in the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content. JVS guided and edited the project article for (3) final approval of the version to be submitted.

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Appendix A. Questionnaire used for study

Study of impact of tobacco on elderly health

Date : _____ Village Name:_____ Form. No: ☐

A. Socio-demographic profile:

1.Name of Elderly: _____

2.Age: ☐ **3. Sex:** (1) Male (2) Female. ☐

4.Religion: (1) Hindu (2) Muslim (3) Sikh (4) Christian (5) Others. ☐

5. Socioeconomic status Scale :

(A) Caste : (1) schedule caste (2) lower caste(3) artisan caste (4) agricultural caste (5) prestige caste (6) dominant caste ☐

(B) Occupation: (1) labour (2) caste occupation (3) business (4) independent profession

(5) Cultivation (6) service ☐

(C) Education: (0) illiterate (1) can read only (2) can read and write (3) primary

(4) Middle (5) high school (6) graduate ☐

(D) Social participation :(1) member of one organization (2) member of >1 organization (3) office holder in such organization (4) wider public leader ☐

(E) Land holding: (0) no land (1) <1 acre (2)1-5 acre (3)5-10acre (4)10-15acres (5)15-20 acre (6) >20 acre ☐

(F) Type of House: (0) no home (1) hut (2) katcha house (3) mixed house (4) pucca house (6) Mansion ☐

(G) Farm power (0) no drought animal (2) 1-2 drought animals (4) 3-4 drought animals or 1 or more prestige animals (6)5-6 drought animal or tractor ☐

(H) Material possession (1) bullock cart (1) cycle (1) radio (1) chairs (2) improved agricultural implements ☐

(I) Family: type- (1) nuclear (2) joint, size- (1) upto 5 (2) above 5, (2)distinctive feature ☐

5. Socioeconomic class:(1) upper class >43 (2) upper middle 32-42 (3) middle 24-32 (4) lower middle 13-23 (5) lower<13 ☐

6. Have you used any kind of the tobacco products in last 6months ? Yes/No ☐

7. If yes, Which of the following Tobacco-products you are using?
(a) Cigarettes (b) bidis (c)Gutkha (d)Khaini (e)hukka (f) Other ☐

08. When do you use tobacco? ☐

(a)When feeling stressed	(b) when wanting to cheer up
(c)When drinking coffee, tea or soda	(d) when feeling anxious
(e)When bored	(f) When wanting something in your mouth
(g) After meals	(h) when at work
(i) When relaxing	(j) when around other users
(k) When at home	(l) with alcohol
(m) while going for defecation	(n) other

09. What was your age when you tried any tobacco products for the first time?

(a) less then 30 (b) 30-40

(c) 40-50 (d) > than 50

10. Does anyone in your family smoke or chew tobacco?

a) **Yes** b) No.

11. Number of tobacco users in your family.

(a) 1 (b) 2 (c) 3 (d) 4 or more

12. Has anyone in your family discussed the harmful effects of smoking with you?
a) Yes (b)No

13. Why did you start use of tobacco?

(a) it is very common Practice. (b) pressure from friends.

(c) Want to look grown up smart. (d) it relives tension.

(e) Just want to try.

14. How harmful do you think is tobacco?
(a) Tobacco use is a leading cause of disease and death

(b) Tobacco use causes some serious illnesses but not death

(c) Tobacco use causes some minor illnesses (d) not know.

15. Have you tried to stop using tobacco before today?

(a) Yes (b) no

16. How many times have you tried to stop using tobacco?

_ 1 _ 2 _ 3 _ 4 _ 5 or more times

17. What is the longest period you have gone without using tobacco?

_____ (hours, *days, weeks, months, or years)*

18. What made you start again?

(a) house hold members who smoke or use tobacco (b) cravings (c) fear of weight gain (d) friends who smoke or use tobacco (e)stress
(f) other……..

19. What is your main reason to quit using tobacco?

(a) Health Reasons (b) To Save Money

(c) To be a Positive Role Model (d) Live Longer

(e) Protect the health of others (f) Advised by doctor/ other

(g) Other

20. Have you been taught about the ill effect of tobacco use in any of your school/ college?

(a) Yes (b) No (c) not applicable.

21. Have you ever received help or advice to help you stop tobacco use?

a) Yes, from a program or professional
b) Yes, from a friend
c) Yes, from a family member
d) Yes, from both programs or professionals and from friends or family members
e) No

22. Are you in favor of banning smoking in public places?
a) Yes b) no

23. How many advertisements or promotions for cigarettes have you seen in newspapers or magazines?
a. A lot b. A few c. None

24. When you come across some tobacco related article in newspaper etc how often do you read the article?

(a) Always (b) Often (c) Seldom (d)I rarely read that type of article.

25. Do you like watching film actors while doing smoking on screen?
(a) A lot (b) Sometimes (c) Never.

26. Do you know that tobacco use is harmful? Yes-1, No-2

a)It causes Lung diseases

b) It causes Cancer

d) It causes death

e) I do not know

Ifyes,how?

_____

27. Do you know that tobacco effects your Oral health? Yes-1, No-2

If yes, how?

_____

28.a) How does tobacco smoke affect the lungs?

b) If you smoke but don't inhale, is there any danger?

c) Does smoking tobacco affect your heart?

29. Physical examination Findings

a) Pulse
b) BP
c) Resp
d) Temp
General Condition_____

30. Provisional Dx_____

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Original Article

A study on knowledge and awareness about tuberculosis in senior school children in Bangalore, India

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ABSTRACT

Background: Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), commonly affecting the lungs. All health care professionals including the pharmacists provide a valuable public health role in promoting community awareness of TB particularly in reducing stigma attached to TB. Thus, creating awareness at a community level could play a vital role in control and prevention of TB.

Objectives: To determine whether educational intervention would affect the level of TB awareness among students of selected schools and pre-university colleges (PUCs) in Bangalore urban and Bangalore rural regions.

Methodology: The present study was conducted among the students of 8th, 9th, 10th and PUC in Bangalore rural and urban jurisdiction ($n = 2635$). A questionnaire was designed in English and Kannada language, consisting of 20 questions with multiple-choice answers. A 30-minute visual health education was given on TB in English, followed by general pictorial presentation, and the data were collected as pre-test and post-test.

Results: Data collected from 2635 participants during pre- and post-education session revealed that mean score improved from 8.77 ± 2.59 to 14.95 ± 1.99. Impact of the education session showed a significant knowledge improvement about TB from 1.59% (pre-education) to 49.67% (post-education).

Conclusion: The present study clearly demonstrated that a simple, 30-minute health education session did have a positive impact on knowledge and awareness about TB among school children as observed with increase in mean knowledge score from pre-test to post-test, indicating that empowerment of students could guide the community on various aspects of TB.

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1. Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis (M. tuberculosis)*, affecting the lungs,[1] producing either a silent, latent infection or a progressive, active disease. Left untreated or improperly treated TB causes progressive tissue destruction and eventually death. *M. tuberculosis* is transmitted from person-to-person by coughing or sneezing, which produces ''droplet nuclei'' that are dispersed in the air, and may contain one to three organisms.[2] In healthy people, infection with *M. tuberculosis* often causes no symptoms, since the person's immune system acts to ''wall off'' the bacteria. The symptoms of active TB affecting the lungs are coughing, sometimes with blood in sputum at late stage, chest pain, weakness, weight loss, fever and night sweats.[1]

TB continues to be a major infectious disease killing more humans than any other disease. One-third of the world's population continues to be infected with tubercle bacilli and eight million new cases appear every year besides two million deaths occurring each year due to TB.[3] The latest estimates included in the Global TB report 2014 indicates that there were 9.0 million new TB cases in 2013 and 1.5 million TB deaths (1.1 million among human immunodeficiency virus (HIV)-negative people and 0.4 million among HIV-positive people).[4]

India is the highest TB burden country globally, accounting for one-fifth of the global incidence and 2/3rd of the cases in southeast Asia. Nearly 40% of the Indian population are infected with the TB bacillus, and India stands out as having the largest number of incident cases in 2013 (2.0 million–2.3 million).[4,5] Each year, 1.9 million new cases of TB occur in the country, of which about 0.8 million are infectious new smear positive pulmonary TB cases. As reported in 2009, estimated deaths due to TB each year are about 330,000, over 1000 deaths a day, which means that 2 deaths occur every 3 minutes in India.[5] The TB situation in the country is further threatened by the emergence and spread of HIV and drug-resistant TB.

Unlike the risk of acquiring infection with *M. tuberculosis*, the risk of developing disease after being infected depends largely on, which includes, the number of *M. tuberculosis* organism's inhaled (infecting dose), the virulence of these organisms, and the host's cell-mediated immune response.[2] Clinical illness directly following infection is classified as primary TB, and is common among children up to 4 years of age, which is severe and disseminated, and it is usually not transmissible. When infection is acquired late in life, the immune system will contain it, at least temporarily. Dormant bacilli may persist many years before reactivating to produce secondary (or post primary) TB, which is often infectious. Overall, it is estimated that ~10% of persons infected in their youth will eventually develop active TB.[6]

The present scenario stresses on the urgent need to view and deal with TB not only as a medical or public health problem but also as a social problem, where innovative interventions have to be taken seriously for its effective control. A country like India requires awareness among all communities for preventing and controlling TB. Most of the population may not be aware about the danger of drug-resistant TB due to irregularity in consumption of anti-TB medication for prescribed period of time. Mere access to health facilities with free anti-TB drugs may not be enough to bring about desired success in preventing and controlling TB.

Pharmacist is the most easily accessible member of the primary health care team, and for the public, and can play a more proactive role in preventing and managing TB towards the patients. Pharmacists have a valuable public health role in promoting community awareness of TB, particularly in reducing the stigma and discrimination often associated with the disease.

In purview of all the above, the objective of the present study was to determine if educational intervention would affect the level of TB awareness among students of selected schools and pre-university colleges (PUC) in Bangalore Rural and Bangalore Urban region.

2. Methodology

The present work was a community-based study conducted in students from randomly selected high schools and PUC) in Bangalore Rural and Urban region, who were interested in and gave consent to participate in the study. The total number of 2635 students from high schools (eight, ninth and tenth standard) and PUCs (first year PUC), aged between 11 and 18 years participated in the study. The purpose of the study was explained to the teachers and students and was assured of the confidentiality of all replies. The complete project was done according to Declaration of Helsinki and approved by the Institutional Ethics committee of V.I.P.S., Bangalore.

2.1. *Data collection*

A questionnaire designed in English and local language (Kannada) was distributed among the students for self-administration. The questions were compiled from ''Avoiding tuberculosis'' self-study programme on TB, a WHO health academy initiative reference. This designed questionnaire was validated by the project co-coordinator of RNTCP (Revised National TB Control Program). The questionnaire contained a total of 19 questions to assess the knowledge of students regarding TB on the following domains: nature of the disease, its mode of transmission, signs and symptoms, diagnosis, about DOTS (Direct Observed Treatment Short Course) program, risk factors, HIV/AIDS (Acquired Immno Deficiency Syndrome) and TB co-infection, vaccination, treatment and prevention. All the questions were objective in nature and the respondents were free to choose any answer from the given options. Following this, a 30-minute visual health education session was conducted on TB in English and Kannada (local language). With a gap of one week following the presentation, the same pre-test questionnaire was distributed among the students for self-administration and recorded as post education data.

Feedback was collected from the validated questionnaire towards pre- and post-education session. The feedback of pre-education and post-education questionnaire had 4 optional answers. Responses to the questions were analyzed based on correctness of information. The knowledge score was given for each participant after pre- and post-education session with a

minimum score as "0" (all answers wrong) and maximum score as "19" (all answers correct).

The knowledge levels were categorized on the obtained marks as poor knowledge (<50%), moderate knowledge (51–75%) and good knowledge (75–100%). The percentage level of knowledge was calculated from the following formula:

$$\text{Percentage (\%) level of knowledge} = \frac{\text{Knowledge score}}{\text{Total no. of questions}} \times 100.$$

2.2. Statistical analysis

A descriptive statistical analysis was used and results obtained from continuous measurements are presented as mean ± standard deviation (SD). Paired *t*-test was used to find the significance of study parameters using the software GraphPad Prism 6 for Windows, Version 6.05.

3. Results and discussion

The participants in the awareness programme comprised of school students from high schools and PUC; their demographic details are depicted in Table 1. A total of 2635 students participated in the pre-education session, in which females constituted about 44.70% (1178) who were found to be less compared to that of males 55.29% (1457); similarly, in the post-education session, male students were higher in number than female students.

A total number of 357 (13.54%) students from 8th standard, 885 (33.58%) from 9th standard, 1113 (42.23%) from 10th standard and 280 (10.62%) students from PUC participated in the study. Hundred and eighty-nine students did not participate in post-education session, as they were on leave when the study was conducted at their respective schools and PU colleges.

Table 1 – Demographic details.

Demographics	Pre-education N (%)	Post-education N (%)
Gender		
Male	1457 (55.29%)	1362 (55.68%)
Female	1178 (44.70%)	1084 (44.31%)
Age (in years)		
11–14	1055 (40.03%)	1020 (41.70%)
15–18	1580 (59.96%)	1426 (58.29%)
Level of education (Standard)		
8th	357 (13.54%)	357 (14.59%)
9th	885 (33.58%)	804 (32.86%)
10th	1113 (42.23%)	1022 (41.78%)
PUC	280 (10.62%)	263 (10.75%)
Total	**2635**	**2446**

PUC, Pre-University College. Students from both English and Kannada (local language) medium of instruction from Bangalore rural and Bangalore urban participated in the study. The total number of students during the post-education session was reduced (*N* = 2446), as they were on leave.

3.1. Knowledge assessment of TB among the study population using the validated questionnaire

3.1.1. Pre-education session

Table 2 gives the detailed list of questionnaire comprising of 19 questions and 4 optional answers. The percentage of students opting the correct response is expressed in the table. Only 1181 (59.70%) were aware that TB is an infectious disease that usually affects lungs, and 1443 (72.95%) knew that TB is caused by *M. tuberculosis*. In a study done in by Koay, to study the knowledge about TB among the people in Malaysia, responses against its mode of transmission (38%) were poor.[7] In our study, for the question regarding the transmission of the TB disease, 1285 (64.96%) respondents were aware that TB is a communicable disease.

In a similar study done by Fitzroy AO in Trinidad among pre-university students to assess the knowledge about TB, 80.2% of them believed that it can spread via coughing and sneezing.[8] In our study, only 1169 (59.10%) students knew that TB spreads through the air when an infected person coughs or sneezes. Few students answered that TB spreads through water and it can spread by sharing food with a person infected with TB.

A total of 1404 (70.98%) respondents were aware that TB commonly affects lung. A study was conducted by Nguyen P.H. in a rural community in Vietnam to evaluate the effect of communication channels towards KAP (knowledge, attitude and practice) on TB, where only few knew about signs and symptoms of TB.[9] Here in our study, 1068 (53.99%) students were aware that cough, fever, night sweat, weight loss, shortness of breath and chest pain were the common symptoms of TB, and 291 (23%) students had an idea that coughing and blood with sputum are the only symptoms of TB.

In a study reported by Aftab on TB and awareness about spread and control, about 8% thought that cessation of smoking can be used in the management of TB.[10] In the present study, the negative perception towards smoking as a cause of TB is high with 61.2% answering that smoking leads to TB, and only 438 (22.14%) agreed that smoking do not cause TB. In the present study, 832 (42.06%) students were aware that sputum AFB (acid fast bacillus) is the initial choice of diagnosis for the treatment of TB. Only 856 (43.27%) students knew that sputum smear microscopy is the accurate method of diagnosis for active pulmonary TB. A total of 785 (39.68%) students were aware that DOTS refer to a method of TB treatment. In a similar study reported by Charkazi AR among medical interns to assess the knowledge on TB and DOTS strategy in northern Islamic Republic of Iran, only 6.3% were familiar about AFB sputum microscopy as diagnostic tool in TB treatment and 16.5% were able to describe about DOTS concept.[11]

A study was carried out in China to assess the gender difference in the knowledge of TB and associated health-care seeking behaviours, where majority were aware that TB is a curable disease.[12] In the present study, the perception towards treatment of TB was positively good; about 1509 (76.28%) students being aware that TB is preventable and 1412 (71.38%) students answered that TB is curable.

In a pilot study conducted by Govt. of Delhi under the supervision of Sharma to study the impact of IEC (Information, Education and Communication) campaign on TB awareness

Table 2 – Comparison of correct responses using a validated questionnaire.

Questions	Response	Pre-education	Post-education
1. What is tuberculosis?	a) TB is a symptom of HIV infection		
	b) TB is a hereditary disease that only affects children		
	c) TB is an ancient disease that has been eradicated.		
	d) TB is an infectious disease that usually affects the lungs	*1181 (59.70%)*	*1446 (80.78%)*
2. Which of the following bacteria causes TB?	a) *Plasmodium falcifarum*		
	b) *Escherichia coli*		
	c) Mycobacterium tuberculosis	*1443 (72.95%)*	*1589 (88.77%)*
	d) Streptococcus species		
3. Is tuberculosis communicable disease?	***a) Yes***	*1285 (64.96%)*	*1671 (93.35%)*
	b) No		
	c) May be		
	d) Don't know		
4. How TB spreads?	a) TB can spread through water		
	b) TB can spread by sharing food with a person infected with TB		
	c) TB can be spread through the air when an infected person coughs or sneezes	*1169 (59.10%)*	*1550 (86.59%)*
	d) TB can be spread through holding hands with an infected person		
5. Which organ is most commonly affected by TB bacteria?	***a) Lungs***	*1404 (70.98%)*	*1661 (92.79%)*
	b) Bones		
	c) Brain		
	d) Liver		
6. Which parts of the body can be affected by TB?	a) TB affects only the lungs		
	b) TB affects only the lungs, bones & joints & brain		
	c) TB affects only the lungs, bones & joints & Urinary tract		
	d) TB affects only the lungs, lymph glands, bones & joints, brain, Urinary tract & reproductive system	*405 (20.47%)*	*1289 (72.01%)*
7. People with pulmonary TB may have the following symptoms?	***a) Coughing, fever, night sweat, weight loss, shortness of breath, chest pain***	*1068 (53.99%)*	*1633 (91.22%)*
	b) Coughing, blood is the only symptom of TB		
	c) Severe headache and weight loss		
	d) Collapse of immune system and development of AIDS		
8. Does smoking cause TB?	a) Yes		
	b) No	*438 (22.14%)*	*1334 (74.52%)*
	c) May be		
	d) Don't know		
9. Sputum AFB Test is the initial choice of diagnosis for the treatment of TB?	***a) Yes***	*832 (42.06%)*	*1372 (76.64%)*
	b) No		
	c) May be		
	d) Don't know		
10. Which one of the following is the most accurate method of Diagnosis for active Pulmonary TB?	a) Discussion with the patient		
	b) Culture test		
	c) Sputum smear Microscopy	*856 (43.27%)*	*1108 (61.89%)*
	d) X-ray		
11. DOTS refer to a method of treatment of TB?	***a) Yes***	*785 (39.68%)*	*1412 (78.88%)*
	b) No		
	c) May be		
	d) Don't know		
12. Is tuberculosis preventable?	***a) Yes***	*1509 (76.28%)*	*1606 (89.72%)*
	b) No		
	c) May be		
	d) Don't know		
13. Is tuberculosis curable?	***a) Yes***	*1412 (71.38%)*	*1668 (93.18%)*
	b) No		
	c) May be		
	d) Don't know		
14. Duration of treatment for TB?	a) 1 month		
	b) 3 months		
	c) 4 months		
	d) 6–9 months	*751 (37.96%)*	*1523 (85.08%)*
15. Which one of the following statements best describes the relationship between HIV/AIDS and TB?	a) HIV/AIDS and TB share a similar mode of transmission: both can be transmitted through unprotected sex		
	b) An HIV infection and TB infection produce similar symptoms, including fatigue, weight loss, and cough		
	c) HIV promotes the progression from latent TB infection to active disease and vice versa	*389 (19.66%)*	*951 (53.12%)*
	d) HIV/AIDS is a symptom of TB		

Table 2 (*Continued*)

Questions	Response	Pre-education	Post-education
16. The average life span of an untreated HIV infected person who contracts TB is	a) 6 days ***b) 6 weeks*** c) 6 months d) 6 years	*408 (20.62%)*	*1174 (65.58%)*
17. Which of the following vaccine is used to prevent TB?	***a) BCG vaccine*** b) Polio vaccine c) TT vaccine d) Hepatitis vaccine	*797 (40.29%)*	*1416 (79.10%)*
18. Which one of the following best describes MDR-TB?	a) An effective drug regimen that can cure active pulmonary TB b) A side-effect of antibiotics used to treat active pulmonary TB and is characterized by nausea and Headaches ***c) A type of TB that is resistant to 2 or more anti-TB drugs*** d) A type of TB that results when anti-TB drugs multiply rapidly within the body	*573 (28.96%)*	*1003 (56.03%)*
19. Which one of the following is the greatest risk factor for development of active TB disease?	***a) AIDS*** b) Recent TB infection c) HIV infection d) Diabetes	*553 (27.95%)*	*1247 (69.66%)*

PUC, Pre-University College. Data represent the comparison of correct response obtained during pre- (n = 1978) and post-education (n = 1790) session. Data regarding the wrong responses are not shown. For students from Kannada medium of instruction, translated version of questionnaire in Kannada was used.

among the general population and symptomatic cases reporting to DOTS centres, there was a significant improvement in knowledge on TB towards post-IEC campaign in the areas such as diagnosis 44.5% and duration of treatment 65.4%.[13] Ironically, in the present study, the perception about duration of TB treatment is low, where only 751 (37.96%) students were aware that 6–9 months is required for the treatment of TB.

The knowledge towards the relationship between HIV/AIDS and TB was very poor; only 389 (19.66%) students agree that HIV/AIDS promotes the progression from latent TB infection to active disease and vice versa. Only a few students, 408 (20.62%), knew that the average lifespan of an untreated HIV infected person who contracts TB is 6 weeks.

In a study carried out in two districts of Pakistan's Punjab province to assess the urban-rural inequalities in KAP on TB, only 43.8% urban and 32.7% rural were aware about BCG vaccination.[14] Previous study conducted by Accredited Social Health Activists (ASHAs) in India reported that ASHAs had good knowledge, favourable attitudes and practices pertaining to TB but observed gaps in the knowledge about BCG vaccination and major symptoms of pulmonary TB.[15] In the present study, about 797 (40.29%) students were aware that BCG vaccine (Bacillus Calmette-Guerin) is used to prevent TB. In a similar study reported by Charkazi AR, only 42.5% were able to define the term Multi-drug-resistant TB (MDR-TB).[11] In the present study, the knowledge about MDR-TB is poor among the students, with 573 (28.96%) being aware that MDR-TB is a type of TB that is resistant to 2 or more anti-TB drugs. Regarding risk factors, only 553 (27.95%) students knew that AIDS is the greatest risk factor for the development of active TB disease.

3.1.2. *Post-education session*

Unlike pre-education session, improved results were obtained following education session. As per Table 2, out of 2446 students, 1446 (80.78%) students were able to recall that TB is an infectious disease that usually affects the lungs. A study conducted in Ethiopia, to know the knowledge and perception of pulmonary TB in pastoral communities, reported that the study participants possessed very poor knowledge (0.24% of participants) about the cause of TB.[16]

There is a significant improvement in recollection level of information about TB by the students for the questions such as bacteria that causes TB (88.77%), its mode of transmission (86.59%), organs that get affected by TB (72.01%) and regarding symptoms (91.22%) of TB such as cough, fever, night sweats, weight loss, shortness of breath and chest pain. A marked difference was observed after post-education session regarding smoking in association with TB, where about 74.52% students became aware that smoking does not cause TB. In a similar study conducted in West Bengal to assess the impact of 'child to family' strategy for health awareness improvement at rural sectors of Paschim, majority of the students obtained poor grades regarding TB, whereas in the post-awareness session the grades were improved.[17]

In a similar study conducted by Vijayaprasad G to assess the impact of a simple educational intervention on the knowledge and awareness of TB among high school children in Vellore, India,[18] a significant raise in the knowledge levels on treatment of TB was seen. In our study, the core message regarding diagnosis, DOTS, duration of treatment, HIV/AIDS and TB co-infection and BCG vaccination was recollected correctly by 61.89%, 78.88%, 85.08%, 53.12% and 79.10%, respectively. The knowledge levels on MDR-TB and risk factors were improved to 56.03% and 69.66%, respectively.

3.2. *Percentage levels of knowledge*

Change in knowledge level on TB was compared between pre-education and post-education session (Table 3). Amongst a total of 2635 participants in the study, 67.66% had poor knowledge on TB. Level of knowledge increased to a greater extent, which was observed with an increase in the number of

Table 3 – Percentage level of knowledge.

Level of knowledge	Pre-education	Post-education
Poor knowledge (<50%)	1783 (67.66%)	233 (9.52%)
Moderate knowledge (51–75%)	810 (30.74%)	998 (40.80%)
Good knowledge (76–100%)	42 (1.59%)	1215 (49.67%)
Total	**2635**	**2446**

PUC, Pre-University College.
Data represent the number of students distributed based on the level of knowledge about TB.

$$\%\text{Level of knowledge} = \frac{\text{Knowledge score obtained}}{\text{Total no. of questions}} \times 100.$$

students possessing moderate and good knowledge levels and steep decrease in poor level of knowledge after the awareness programme. Among the participants, the number of students having poor knowledge was reduced to 9.52% during the post-education session. Impact of the education session showed a significant improvement from 1.59% (pre-education) to 49.67% (post-education) of students possessing good knowledge.

3.3. *Impact of educational session based on knowledge score*

Educational intervention was found to be effective which showed an improvement in the knowledge scores. The knowledge scores were given for each participant towards pre- and post-education session, with a minimum score as "0" and maximum score as "19". In pre-education session, the mean score ± SD was found to be 8.77 ± 2.59. In post-education session, the mean score ± SD increased to 14.95 ± 1.99 (Fig. 1).

The comprehension and recall of pictorial presentation was done manually by selecting the students randomly. Along with the questionnaire form, the pictogram hand-outs were distributed to the students. By selecting randomly, students were questioned about the pictograms by pointing at them individually. Most of the students were able to recollect the simple information from pictograms on TB such as its mode of transmission, symptoms, DOTS and prevention. Some of them were unable to recollect the information about diagnosis from the pictograms.

The present work revealed that a simple health education session on TB has shown great impact on knowledge and awareness about TB among school children. Hence, educating students would empower them to guide the local community to a vast extent.

4. Conclusion

TB is an infectious disease caused by *M. tuberculosis*; it continues to be a major infectious disease killing more humans than any other disease.

Our study has concentrated on creating awareness about TB among the students. As the current scenario indicates, there is urgent need to view and deal with TB not only as a medical concern or even public health problem alone, but also as a social problem. Hence, educating students would empower them to guide the local community to a vast extent.

As a part of health care system, we, as professional pharmacists, can play a more proactive role in preventing and managing TB in the society, and the foremost being creating awareness among the children as one of the effective

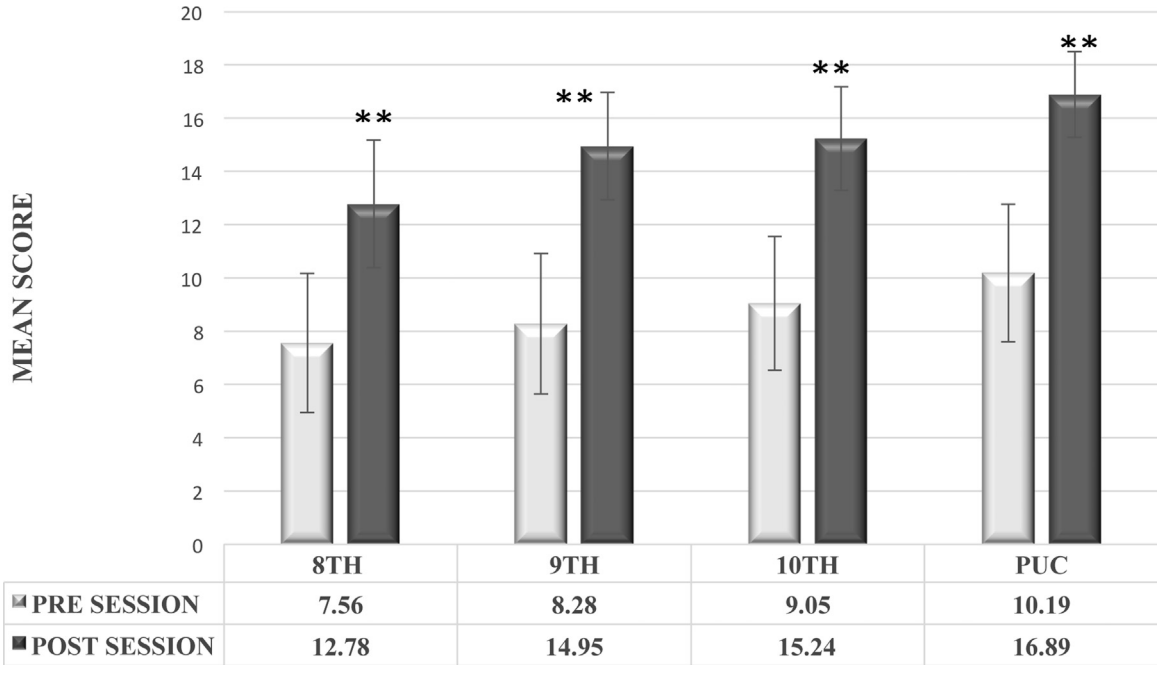

Fig. 1 – Impact of educational session based on mean knowledge score. Data represented as Mean ± standard deviation. Knowledge scores were given for each participant towards pre- and post-education session, with a minimum score as "0" and maximum score as "19". Paired *t*-test was used to determine the level of significance between pre- and post-education data. ** ($P < 0.001$) indicates the significant difference in the responses obtained from pre- and post-education session.

ways of preventing TB. The present study clearly demonstrated that a simple, 30-minute health education session, in the form of multimedia education, did have an impact on students, and this knowledge of students may be used in an effective manner for creating awareness in the community for its involvement in TB control.

The educational module could potentially be replicated in all schools in TB-endemic countries. School children could be empowered to be the flag bearers in the fight against TB.

Conflicts of interest

The authors have none to declare.

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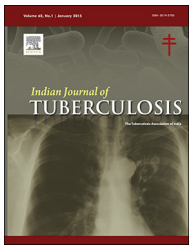

Short Communication

Lessons learnt from active tuberculosis case finding in an urban slum setting of Agra city, India☆

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ABSTRACT

Active case finding (ACF) is recognized as one of the key strategies to reach the missing 3 million cases in high tuberculosis (TB) burden countries. In India, we conducted ACF as a pilot project to assess its operational feasibility in four slums of Agra city in 2012 and covered 3940 households (in 14 wards) with a population of 21,870. Trained community volunteers visited households with an intention to provide information on TB and refer those with cough ≥2 weeks for sputum smear examination.

Volunteers identified 8 persons with cough of ≥2 weeks by asking the first or the main respondent of the household. However, by directly asking (or probing) all available members of the household, they identified 374 persons with cough of ≥2 weeks. All 382 persons with cough of ≥2 weeks were referred for sputum smear examination. While 40% of those referred reached health facilities for sputum examination on their own, 60% had to be accompanied by the community volunteers to the health facility for sputum smear examination by Ziehl–Neelsen staining method. Eventually, seven persons were found to be sputum smear positive. This study highlighted important aspects for implementing ACF: First, all household members have to be asked for TB symptoms and Second, mere referral for sputum examination is not enough and there is a need to support people to reach the health facility for sputum smear examination.

1. Introduction

Early diagnosis and treatment is critical for providing optimal care to tuberculosis (TB) patients and also to prevent its spread in the community. Globally, an estimated one third of the 9.6 million incident cases are either undiagnosed or diagnosed but not notified to public health authorities.[1] Active case finding (ACF) is therefore, one of the key recommended strategies to ensure early diagnosis and link patients to appropriate diagnostic and TB treatment services that are available under the National TB Programmes in high TB burden countries.[2] In India, there has been over reliance on passive case finding; i.e., diagnosing TB among those

☆ The findings were presented in ITM Colloquium on Health Systems and Control of Neglected Diseases in Asia, 21st–22nd November, 2013 in Bangalore.

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presenting to the health facilities. This passive case finding strategy was earlier believed to detect significant proportion of incident TB cases that is necessary for its control while being less burdensome on health system in a resource constrained environment. However, recent studies have shown that this strategy could have contributed for delay in diagnosis of TB and continued transmission.[3,4] Therefore, Government of India currently recommends ACF in marginalized and vulnerable communities.[5] However, the mechanisms for implementing ACF were not clear and hence we undertook a feasibility study in the slums of Agra to understand the operational challenges in its implementation. In this study, we report on (a) the health care providers from which the slum population seek medical care commonly, (b) the results and key lessons learnt in implementing active TB case finding in this setting.

2. Methods

Agra district with a population of 4.5 million is one of the most populated districts of India. Nearly one-third of the population resides in the urban areas. 'Agra city' has 0.5 million slum population distributed in about 90 wards (average number of households per ward is 2900 with ~17,000 populations) [http://southasia.oneworld.net/resources/indias-urban-poverty-in-agra-slums]. In 2013, 5955 smear positive TB patients were diagnosed from the entire district under the National TB Programme (NTP).[6]

Agra is also one of the 300 districts where Global Fund supported Project Axshya is being implemented by The International Union Against Tuberculosis and Lung Disease (The Union) to enhance access to TB services in the vulnerable and marginalized populations (http://www.axshya-theunion.org/). Of the 417 slums in the city, four slums that were reporting the highest number of TB cases were purposively selected in consultation with district level authorities to pilot test the feasibility of implementing ACF.

Ten community volunteers of a local NGO who were working with the national health programmes in these slums as USHA (Urban Social Health Activist) were provided one day training to undertake the following activities: visit house to house, provide information about TB (symptoms, mode of transmission, diagnosis, treatment and availability of TB services), collect demographic details of the household members and ask if any of them have cough of ≥2 weeks. Volunteers initially asked the main or the first respondent of the household about the presence of cough of ≥2 weeks in them and in the other members of the household. Thereafter, they individually contacted all other available household members and asked them about the presence of cough of ≥2 weeks. All persons with cough of ≥2 weeks were line listed and referred to the nearest public health facility for sputum smear examination. If the line listed persons did not visit the health facility on their own for up to a week, then community volunteers revisited the households and accompanied them to the public health facility.

The volunteers were paid an honorarium of INR 10 per household visited, with additional incentives for accompanying people to the health facility to undergo sputum smear examination. This activity was supervised by supervisors from the project Axshya and Senior TB supervisors from the Revised National TB control programme. This activity was conducted from August to October 2012 under routine programmatic conditions in consultation with the district health authorities. The data was entered in an excel sheet and analyzed using SPSS 11.0 version software.

3. Results

A total of 3940 households were visited which comprised of 10,594 adult population aged 18 years and above (5374 males and 5220 females) and 11,276 persons (aged less than 18 years) with a mean household size of five persons.

Nearly 92% of household respondents mentioned that they approached a nearby private healthcare provider in case of any illness and 78% of these providers were within 0.5 KMs of their residence. More than 90% of the household respondents had heard of TB, and in those who had heard of TB, almost all knew that TB is curable and that cough of ≥2 weeks is a symptom of TB (Table 1). By asking the main or the first respondent of the household, the volunteers identified 8 persons with cough of ≥2 weeks. However, by directly asking/probing all available members of the household they identified 374 persons with

Table 1 – Awareness about TB in four slums of Agra city (India) and the number of persons with pulmonary TB symptoms (cough of ≥2 weeks) identified through active case finding, 2012.

Number (%)who had heard about TB (Total respondents *N* = 3940)	3567 (90.5)
Number (%)who knew that cough of ≥2 weeks could be TB (Total respondents *N* = 3567[a])	3564 (99.9)
Number (%)who knew that TB is curable (Total respondents *N* = 3567[a])	3560 (99.8)
Number (%) who mentioned having a member with TB in their household? (Total respondents *N* = 3940)	17 (0.4)
Number (%) who mentioned a family member to be having cough of >2 weeks (response by the first/main respondent) [Total respondents = 3940]	8 (0.2)
Presumptive TB persons identified following probing each available member of the household by the community volunteer	374
Total presumptive TB persons identified in the study	382
Total sputum smear positive patients identified in the study	7

[a] Includes only those respondents who had heard about TB.

Table 2 – Age and gender distribution of persons with pulmonary TB symptoms (cough ≥2 weeks) and the sputum smear positive TB patients diagnosed through active case finding in 4 slums of Agra City, India.

Age group	Men	Women	Total	Sputum smear positive[a]
≤20	59	70	129	Nil
21–40	59	75	134	Nil
41–60	40	54	94	6
≥61	11	14	25	1
Total	169	213	382	7

[a] 6 = (3 men, 3 women), 1 = (1 women).

cough of ≥2 weeks (Table 1). All 382 persons with cough of ≥2 weeks thus identified were referred for sputum smear examination. While 40% of those referred reached health facilities for sputum examination on their own, the remaining 60% had to be accompanied by the community volunteers to the health facility for sputum examination. By ensuring all 382 persons underwent sputum smear examination, seven persons with sputum smear positive pulmonary TB were identified (Table 1). Women constituted 56% of the 382 persons with cough of ≥2 weeks and 4 out of the 7 TB cases identified (Table 2).

4. Discussion

This study shows that the people living in Agra slums predominantly seek medical care from the private health care providers whose qualification and expertise to diagnose and treat TB is unknown and that passive case finding at public health facilities in this situation is unlikely to result in early detection of TB.

From the perspective of designing and implementing ACF, this study highlights three important aspects: First, community volunteers can be roped in with incentives to conduct ACF. Though we did not systematically study its acceptability among various stakeholders, we did not get any complaints from anyone on any aspect while implementing this activity and therefore it seems that this activity is acceptable to the community volunteers, households, TB control Programme staff and staff of the health care facilities. Second, all household members have to be asked (or probed) for TB symptoms and just asking one or two members may not help in identifying all persons with TB. Third, after identifying persons with pulmonary TB symptoms, mere referral for sputum examination is not enough and there is a need to support people to reach the health facility for sputum smear examination. The support could be in terms of the volunteer accompanying the patients to the health facilities or it could be collection and transportation of sputum from the patients to the health facilities. If volunteers have to do either or both these activities then additional incentives have to be built in.

This study also shows that awareness regarding TB and its symptoms were high with more than 90% of the households visited being aware that cough of ≥2 weeks could be TB. These findings are in line with a knowledge, attitude, practices study conducted at about the same time which reported 88% to have heard about TB and 81% had knowledge that cough of ≥2 weeks could be TB.[7] The high awareness levels could be due to exposure to TB messages on mass media such as television and information provided by community-based-health workers.

Despite such high levels of awareness, volunteers were able to identify and refer 382 persons with ≥2 weeks of whom seven were sputum smear positive and had not yet sought medical care. While we did not ascertain the reasons for this, other studies which have looked into this aspect have identified both health system and patient related factors for this.[8,9] Some of these factors could be overcome by implementing ACF along with passive case finding so that patients get appropriate care in a timely manner.

5. Limitations

First, we undertook this activity in a small number of purposively selected slum wards with high TB notification rates and therefore the results and challenges that we found may or may not be generalizable to other slum settings elsewhere. Second, we did not collect information on household characteristics such as socio-economic status and main (or first) respondent characteristics such as age, sex, educational status, occupation or the position in the family, because of which we are unable to analyze and report association between these characteristics and knowledge levels. Third, we did not collect information on why patients did not visit health facilities when referred by community volunteers. In retrospect, we feel that we should have collected that information to better understand this aspect. Fourth, while community volunteers reported that they screened all household members for TB symptom, we did not cross-check the accuracy of this statement in a random representative sample of the households. We feel that the number of TB symptomatics identified is an underestimate, but we are unable to quantify this. Lastly, we did not follow-up on patients who were initially sputum smear negative to assess whether they were subsequently symptom free or diagnosed with sputum smear negative pulmonary TB or other diseases. Therefore, the number of TB cases detected, could be an underestimate.

6. Conclusion

The study highlights a few important operational aspects that may help in designing and implementing ACF in India. We recommend that ACF can be conducted using trained community volunteers, and while conducting ACF efforts must be made to screen/probe all members of the household for TB symptoms and incorporate additional measures to bring patients or their sputum samples to diagnostic facilities.

Conflict of interest

The authors have none to declare.

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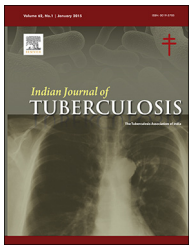

Case Report

Tubercular osteomyelitis of calcaneum bone: A rare occurrence

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ABSTRACT

In spite of the endemic nature of tuberculosis in India, skeletal tuberculosis is relatively infrequent. Involvement of foot bones is uncommon and isolated calcaneum is even rarer. Osteoarticular tuberculosis is a diagnostic enigma, as the characteristic signs and symptoms of this disease may be absent, or mimic other disorders, leading to emergence of complications and therapeutic delay, particularly when the disease affects unusual sites. Here, we are reporting the case of 20-year-old male, who presented with a rare localization of tubercular osteomyelitis involving the calcaneum without adjacent joint involvement to draw attention to this exceptional location in adults, managed with anti-tubercular treatment and gained excellent recovery.

1. Introduction

Despite many advances in diagnosis and management of tuberculosis (TB), it still remains a major infectious disease worldwide. While pulmonary tuberculosis is the most common presentation, extra-pulmonary tuberculosis is also an important clinical problem. It can involve virtually any organ system in the body with osteoarticular involvement ranging from 1 to 3%.[1] Most common site for bone tuberculosis is spine followed by weight bearing major joints like hip and knee joint, and involvement of foot bones is even rare, where it involves calcaneum, talus, 1st metatarsal, and navicular bones in order of decreasing frequency.[2] Isolated calcaneum bone tuberculosis is very rare and only few cases have been reported even from countries like India where tuberculosis is rampant.[3] The calcaneal localization without adjacent joint involvement like in our case is extremely rare, and diagnosis is often missed till late stage of the disease.[4] Foot bone tuberculosis including calcaneum TB may become significantly debilitating if diagnosed late and left untreated. Early diagnosis can omit the need of surgical intervention.[4,5] Here, we are discussing a case of calcaneum bone tuberculosis, diagnosed early and treated with anti-tubercular drugs without any untoward sequel.

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2. Case history

A 20-year-old male patient presented to orthopedics department with complaints of swelling over the lateral aspect of right ankle joint, pain and difficulty in walking for 2 months, fever on and off for 1 month, and lethargy of 20 days duration. His past medical and surgical history was not significant. Physical examinations revealed no abnormality except a swelling over right ankle joint, approximate size of 2 × 2 cm, tender and fixed to underlying structure. Chest skiagram and all routine blood investigations were normal. X-ray anterior-posterior and lateral view of right ankle joint showed an osteolytic lesion in posterior aspect of calcaneum with sclerotic and focal irregular margin, likely osteomyelitis focus (Fig. 1). Magnetic resonance imaging (MRI) of right ankle joint showed rim enhancing area involving medial aspect of right calcaneum with cortical break and adjacent soft tissue extension into right flexor retinaculum, quadratus plantae, and subcutaneous region suggestive of osteomyelitis (Fig. 2). Excisional bone biopsy was done, which on histopathological examination revealed clusters of epitheloid macrophages with Langerhans type of multinucleated giant cells and granuloma, overall finding favoring tubercular pathology (Fig. 3). The culture of the biopsy specimen over Lowenstein–Jensen media for AFB was negative. Clinico-radiological and histopathological examination established the diagnosis of tubercular osteomyelitis. The patient received four drug anti-tubercular

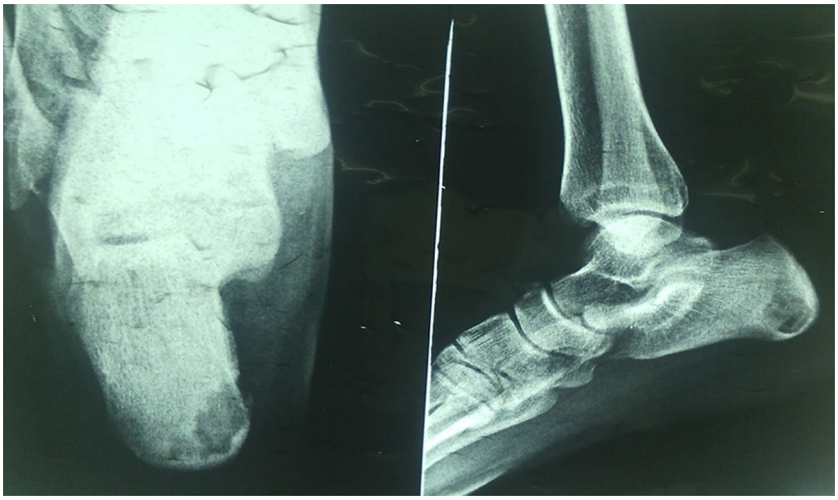

Fig. 1 – X-ray, AP & lateral view of right ankle joint showing an osteolytic lesion in posterior aspect of calcaneum with sclerotic and focal irregular margin.

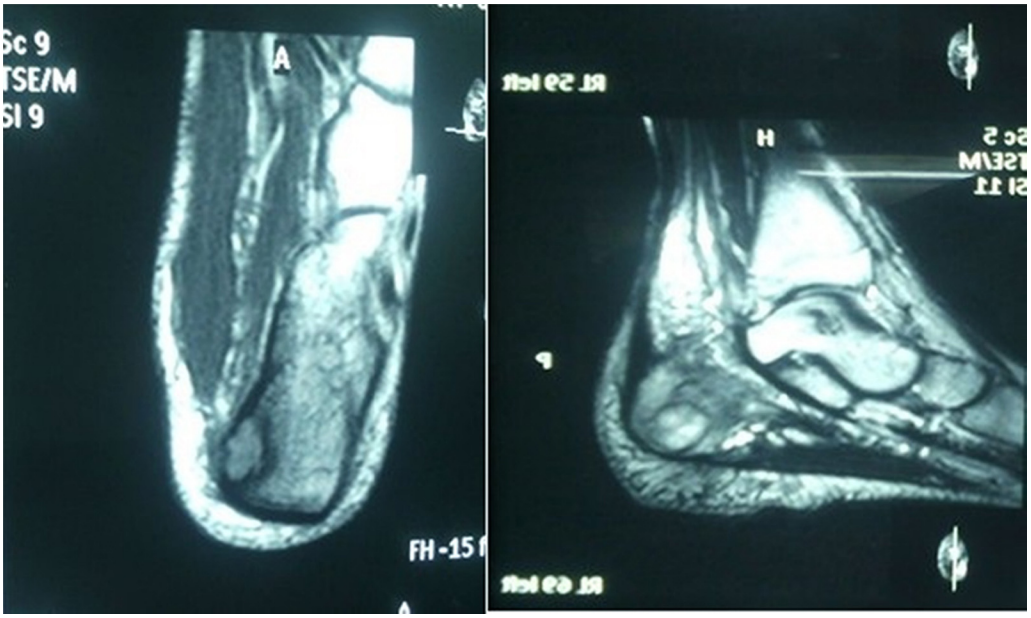

Fig. 2 – Magnetic resonance imaging (MRI) of right ankle joint showing rim enhancing area involving right calcaneum medial aspect with cortical break and adjacent soft tissue extension into right flexor retinaculum, quadratus plantae, and subcutaneous region suggestive of osteomyelitis.

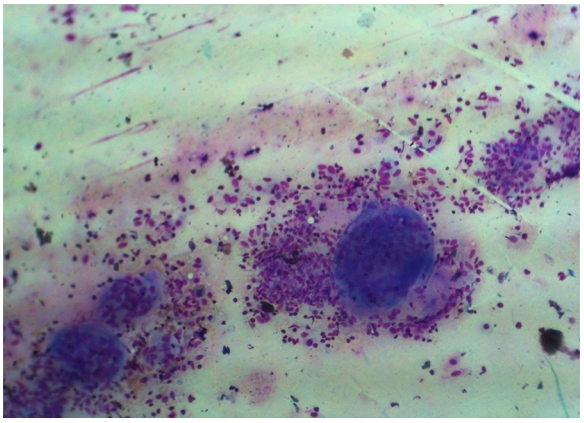

Fig. 3 – Histopathological examination showing clusters of epitheloid macrophages with Langerhans type of multinucleated giant cells (10×).

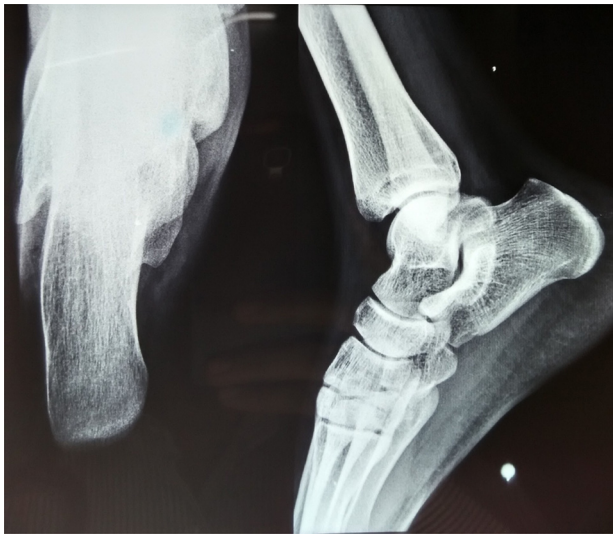

Fig. 4 – X-ray, AP & lateral view of right ankle joint showing an almost complete resolution of osteolytic lesion in posterior aspect of calcaneum.

therapy (isoniazid, rifampicin, ethambutol, and pyrazinamide) and at the end of the treatment, he showed an excellent symptomatic improvement with no pain or limp with full weight bearing on the affected side and an almost complete resolution of lesion on X-ray (Fig. 4).

3. Discussion

Although osteoarticular tuberculosis is uncommon, even in places where tuberculosis is endemic, it has still shown an increased incidence in recent times.[6] Usually, skeletal tuberculosis involves joints and their participating bones, but isolated tuberculous osteomyelitis without joint involvement commonly occurs in the ribs, metacarpals, metatarsals, pelvic bones, skull, sternum, and rarely in large tubular bones. Such isolated osteomyelitis is usually seen only in the early stages of the disease process.[7] The classic presentation with localized pain, swelling, and difficulty in walking together with fever and weight loss is rarely seen in such cases. Tuberculosis of bone may escape the diagnosis for a long time until involvement of a neighboring joint or cold abscess formation presenting as a soft tissue swelling. Tuberculosis of the bone may mimic other similar clinical conditions like chronic osteomyelitis, actinomycosis and madura mycosis, multiple myeloma, or secondary malignant deposits.[8] Radiological features of musculoskeletal tuberculosis are non-specific, but may include bone marrow edema, osteoporosis, or lytic lesions. The surrounding tissue may show synovitis, joint effusions, tenosynovitis, soft tissue collections, or myositis.[9] In calcaneal tuberculosis, commonly a cystic lesion in the middle of the bone without sequestrum formation is found, but a bone sclerotic appearance can also be observed. MRI appearances are non-specific and may be consistent with osteomyelitis, bony tumor, avascular necrosis, or a neuropathic joint, but if tuberculosis is a possibility, a tissue diagnosis should be sought.[10] Synovial fluid aspirate is relatively unlikely to lead to definitive diagnosis and a bone biopsy should also be taken for microscopy, culture, and histology.[2] Tuberculous bacilli are rarely seen (on Ziehl-Neelsen's staining) or grown in culture due to the paucibacillary nature of the biopsy material and the diagnosis often has to be made based on the granulomatous appearance on histology along with high clinical suspicion.[7] Several treatment modalities are available: Anti-TB drugs only, or in addition aspiration of the abscess, open drainage of cold abscess and removal of granulation tissue, curettage of the bony lesion, partial resection of the sternum, and partial resection with reconstruction.

To conclude, calcaneal tuberculosis usually presents a diagnostic challenge as it is less common and, therefore, less familiar to most clinicians. The lack of awareness and rarity of the lesion with its atypical presentation (less dramatic signs and symptoms than osteomyelitis of long bones) make the diagnosis of calcaneum TB very difficult based on clinico-radiological ground, often leading to a delay in diagnosis and management leading to the emergence of debilitating complications. Moreover, synovial fluid aspirate is relatively unlikely to lead to definitive diagnosis and a bone biopsy should always be taken for culture and histopathological examination. In such cases of tubercular osteomyelitis without joint involvement, early diagnosis and appropriate therapy is crucial for a better outcome as in our case and to prevent joint involvement leading to compromised long-term results.

Conflicts of interest

The authors have none to declare.

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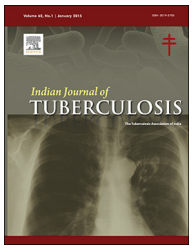

Case Report

Adolescent with recurrent tuberculosis: Can it be chronic granulomatous disease?

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ABSTRACT

Chronic granulomatous disease (CGD) is an inherited primary immunodeficiency disorder with recurrent bacterial and fungal infections like *Staphylococcus aureus*, *Nocardia* spp, *Serratia marcescens*, *Burkholderia cepacia*, *Salmonella* spp. and *Aspergillus* species. We present a 13-year-old male child who had 3 episodes of tuberculosis (TB) at 5 years, 8 years and 13 years of age, respectively, with no other intercurrent infections and who was diagnosed as CGD at the age of 13 years. This case highlights the possibility of phenotypic variations of CGD. The diagnosis of CGD should also be sought in all children with recurrent TB.

1. Introduction

Chronic granulomatous disease (CGD) is a rare inherited primary immunodeficiency which renders patients susceptible to severe, recurrent life-threatening bacterial infections such as enterobacteriaceae, staphylococcus, nocardia, atypical mycobacteria and fungal infections such as aspergillus and candida with majority of patients being diagnosed in early infancy.[1] However, some patients might present in late childhood or early adulthood with recurrent and unusual infections.[1] Most patients would require bone marrow transplant (BMT) in early childhood as the mean survival rate reported in India without transplant is around 1.5 years.[2] We present a boy with recurrent tuberculosis (TB) who was diagnosed to have CGD during his 3rd episode of TB at the age of 13 years and who has survived without a BMT suggesting that there could be phenotypic variations in CGD.

2. Case report

A 13-year-old male child, 1st of twin [the other twin died at 2½ years of age due to TB (details not available)] born of non-consanguineous marriage, presented in September 2014 with cough and fever 20 days ago which lasted for 10 days. The child was currently asymptomatic but had lost 3 kg in the present illness. On examination, weight was 24.8 kg and he had right inframammary crepts. Other systems were normal. He had a histopathological proven tuberculous osteomyelitis of the rib at 5 years for which he was treated with 9 months of first-line anti-tubercular treatment (ATT). At 8 years of age, he again had histopathological diagnosed cervical lymph node TB for which he again took first-line ATT for 9 months. He had no other infections in between. Investigations during current episode showed right lower zone and perihilar haziness on chest X-ray. HRCT chest showed patchy consolidation in both lungs with

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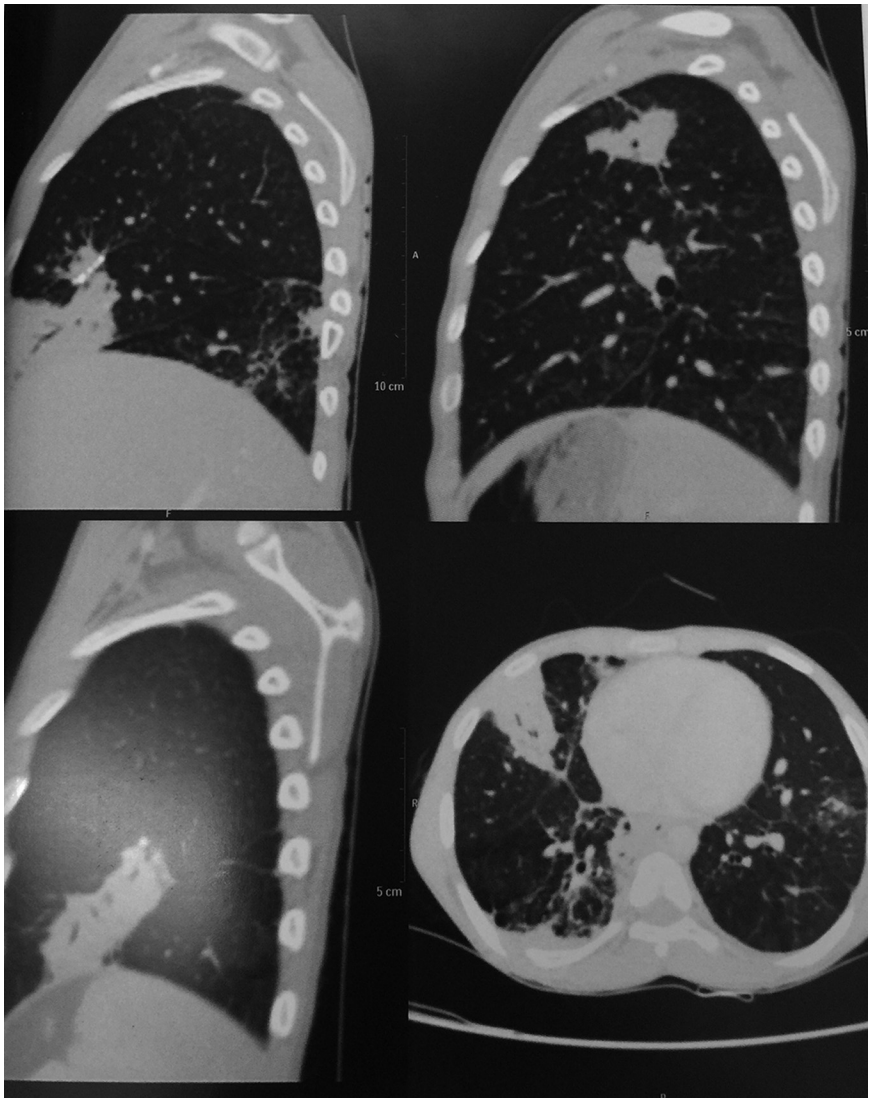

Fig. 1 – CT chest showing air bronchogram in right middle lobe, with partly necrotic nodes in subcarina, right hilar and pretracheal region.

air bronchogram in right middle lobe, with partly necrotic nodes in subcarina, right hilar and pretracheal region (Fig. 1) suggestive of TB. Sputum for acid fast bacillus and TB culture was negative. HIV Elisa was negative. In view of recurrent TB, he was clinically suspected to have an underlying immunodeficiency. Serum immunoglobulin levels [IgG: 33.2 g/dl (3.5–16.2 g/dl), IgA: 10 g/dl (1.50–3.83 g/dl), IgM: 2.42 g/dl (1.44–3.93 g/dl) and IgE: 58.3 IU/ml (3–423 IU/ml)] and lymphocyte subset analysis [CD4: 1163 (26%) (530–1300), CD8: 1923 (43%) (330–920), CD3: 3488 (78%) (1000–2200), CD19: 402 (9%) (110–570), CD16/56: 537 (12%) (70–480)] were normal. Nitroblue tetrazolium (NBT) test was 0% and flow cytometry using dihydrorhodamine (DHR) was 1.5% (normal limits 95–100%) confirming the diagnosis of CGD. As TB recurred after a gap of 5 years, drug-resistant TB was less likely and he was started on first-line ATT consisting of isoniazid, rifampicin, ethambutol and pyrazinamide along with cotrimoxazole prophylaxis. He is on regular follow-up.

3. Discussion

CGD is a rare inherited primary immunodeficiency, which renders patients susceptible to severe, recurrent life-threatening bacterial and fungal infections.[1] It was first described in 1959 and was known as fatal granulomatous disease of childhood.[3] Inheritance is usually X-linked (XL), but can be autosomal recessive (AR).[4] Incidence is 1 in 2,00,000 and 1 in 2,50,000 live births.[5] The actual incidence is likely to be higher due to under diagnosis of patients presenting with milder disease phenotype.[2] CGD is caused due to mutations in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex that leads to an inability of phagocytes to undergo effective respiratory burst, thus resulting in impaired killing of intracellular microorganisms.[6] Most patients present with purulent bacterial infections (such as pneumonias, sinusitis or liver abscess) or necrotizing fungal infections of

deep tissue or bone.[1] Mycobacterial infections both due to BCG and *M. tuberculosis* have also been reported.[7,8] Individuals with XL-CGD are said to have a more severe clinical phenotype and increased mortality than those with AR CGD.[9] In a study in north west India, the mean age of survival without stem cell transplantation was 1 years 5 months [mean survival in XL-CGD was estimated to be 31.23 months (95% CI: 5.1, 57.8 months), while the mean survival in AR-CGD was 176.6 months (95% CI: 72.2, 281 months)].[2] The oldest reported age of CGD is 69 years.[10] CGD may present at any time from infancy to late adulthood, but the majority of patients are diagnosed by the age of 2 years.[11] In a study conducted in Saudi Arabia, the oldest patient was diagnosed at age 17 years and the oldest patient alive was 22 years of age.[6] Our patient had no history of infections till 5 years of age and only had recurrent TB. In fact, a diagnosis of CGD was made only at the age of 13 years after he had these recurrent infections. A growing number of patients are diagnosed in later childhood or adulthood. This is due in part to recognition of milder cases of AR CGD, as well as delayed diagnosis in some patients. Diagnosis may be delayed because of potent antimicrobials that inadvertently treat many CGD-associated infections, postponing diagnosis until more severe infections indicate CGD as the underlying cause. The functional diagnosis of CGD can be made by demonstrating the inability of phagocytes to produce a normal respiratory burst. This is done by the phorbol myristate acetate-stimulated NBT[12] test. In this test, incubation of activated neutrophils with the yellow dye NBT results in the accumulation of dark blue pigment, formazan, within normal phagocytes. The other means of diagnosing CGD is the DHR flow cytometric test, which relies on the reduction of DHR by stimulated phagocytes in heparinized whole blood and provides a quick and convenient method for semiquantitatively determining NADPH ± oxidase function.[13]

Management involves prompt treatment of infections using appropriate antimicrobials. Prophylactic antibacterial and antifungals are advised. Immunomodulatory treatment with interferon gamma can be used for prophylaxis for infections. In select situations, granulocyte transfusions may be required. Definitive treatment remains haematopoetic stem cell transplantation (HSCT).[1] Whether HSCT would be required in all patients with CGD is debatable, as our patient seems to have a milder phenotype of the disease.

4. Conclusion

Patients with CGD may have a variable clinical phenotype. CGD should be suspected in patients with recurrent TB. Not all patients with CGD may require BMT.

Conflicts of interest

The authors have none to declare.

Authors' contribution

Dr. Yashashree Gupta and Dr. Ira Shah: Acquisition of data, analysis and interpretation of data. Drafting of article and revising it critically for important intellectual content. Final approval of version submitted.

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Case Report

Renal tuberculosis presenting as acute pyelonephritis – A rarity

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ABSTRACT

One of the major health problems faced particularly by the developing world since ages is that of tuberculosis (TB). Genito-urinary tuberculosis (GUTB) is the second most common extrapulmonary TB, with kidney being the most frequent site of infection. Due to the diverse and atypical clinical manifestations of urinary TB, the disease is easy to misdiagnose. The diagnosis of renal TB should be suspected in a nonspecific bacterial cystitis associated with a therapeutic failure or a sterile pyuria and a past history of pulmonary TB with important radiologic findings, particularly with the help of CT scan. Here, we describe a case of renal TB where no clinical or radiological features suggestive of renal TB were present. The diagnosis was only evident after the histopathological examination of the excised kidney. This case highlights the importance of suspecting renal TB as an important cause of kidney disease, which can lead to irreversible renal function loss particularly in an endemic area, and also the diversity that this disease may acquire in its presentation leading to misdiagnosis. In such a case, particularly in a high endemic area for TB, therapeutic trial of ATT may also be considered to avoid unnecessary surgical intervention and end-stage renal disease.

1. Introduction

Genito-urinary tuberculosis (GUTB) is the second most common extrapulmonary tuberculosis (TB),[1] with kidney being the most frequent site of infection.[2] Renal TB usually presents with dysuria, hematuria, flank pain, and azotemia, along with constitutional symptoms of fever and weight loss. Most of the times, diagnosis is obviously based on the presence of these symptoms, along with urine examination for acid-fast bacilli (AFB) and mycobacterial (MTB) culture and CT scan of the abdomen and pelvis.[2,3]. Rarely GUTB presents as an acute pyelonephritis leading to pyonephrosis and end-stage renal disease (ESRD), ultimately paving the pathway for nephrectomy.[4–6] We are presenting a case of renal TB presenting as acute pyelonephritis.

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2. Case

A 23-year-old female, nondiabetic and nonhypertensive, presented to us with complaints of high grade fever with chills and right upper abdominal pain radiating to the hemithorax for 15 days. There was no history of dysuria, hematuria, or recurrent urinary tract infections. She was referred by a general practitioner suspecting her to be suffering from pneumonia along with pleural effusion. But on evaluation, the respiratory examination came out to be totally within normal limits. Other than tachycardia and elevated temperature, the rest of the physical examination was also normal. Keeping possibility of probable infectious pathology in mind, detailed radiological and hematological examination was undertaken to find out the cause and site.

Chest radiograph was normal (Fig. 1), as well as the routine blood investigations. Ultrasound examination of abdomen revealed cholelithiasis with mild hydroureteronephrosis (HDUN) with suspicion of infected HDUN. Mantoux test showed an induration of 5 mm. Urine examinations showed pyuria. Urine analysis for AFB was negative on 3 consecutive days. Urine culture showed no growth of MTB. There was no history of previous treatment for pulmonary TB. As no evidence of pulmonary TB or pneumonia was found in the patient, she was referred to the department of urology for further management and ruling out possible acute pyelonephritis.

A renal dynamic scan was done there, which showed right hydronephrotic kidney with impaired parenchymal function. This was followed by drain placement and right anterograde pyelography (Fig. 2), revealing a stricture in right upper ureter. As a double J stenting could not be done, a percutaneous nephrostomy (PCN) was placed and thick pus was drained. The pus showed no AFB, and showed growth of nonpathogenic organisms on pyogenic culture. Due to negligible urine output from the PCN, a repeat renal dynamic scan was performed, which showed deterioration in parenchymal function, as well as reduction in the size of the right kidney. CT scan of lower abdomen and pelvis (Fig. 3) was performed, which revealed right-sided hydronephrosis with collection within the calyceal system and poor renal function. So, right nephrectomy was planned, taking it to be an infected bacterial hydronephrosis. The resected specimen revealed tuberculous granulomatous inflammation (Fig. 4). The patient was initiated on antituberculous treatment (ATT). The patient was started on the four-drug regimen of ATT, including rifampicin (R), isoniazid (H), ethambutol (E), and pyrazinamide (Z), for 3 months followed by a three-drug ATT regime (RHE) for another 5 months. The patient is in regular follow-up in both the pulmonary and urology departments and is now asymptomatic and doing well since then.

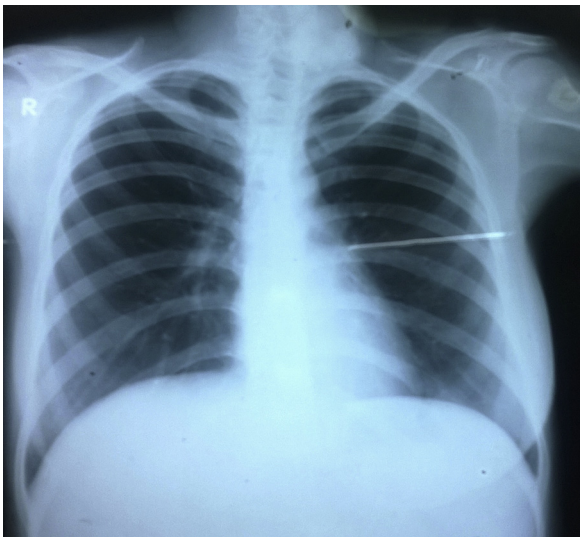

Fig. 1 – Chest radiograph PA view showing no abnormality.

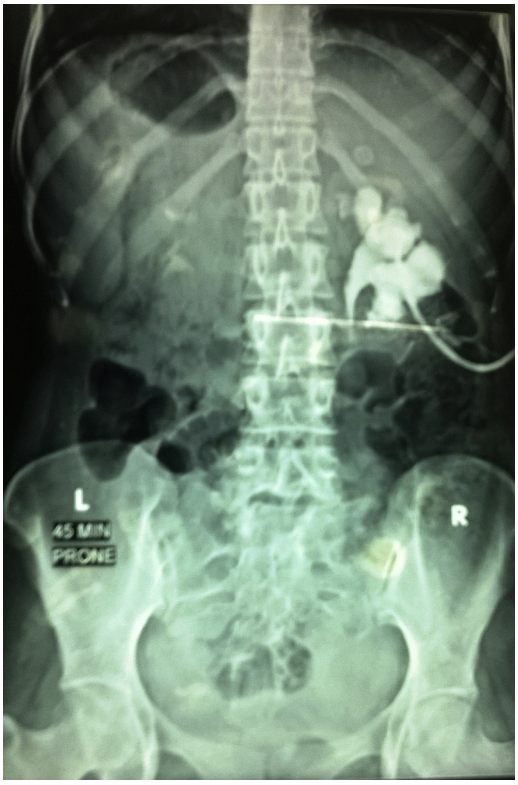

Fig. 2 – Right anterograde pyelography revealing a stricture in right upper ureter.

3. Discussion

Extrapulmonary TB comprises 20–25% of the whole burden of TB.[7] Second most commonly affected site in extrapulmonary TB after lymphadenitis is the genito-urinary system having the contribution of about 4%.[1,8] The most frequent organ involved in GUTB is the kidney (~70%).[5] The usual manifestations of TB that is fever, weight loss, and night sweats may be absent in GUTB. The clinical manifestations of urinary TB are nonspecific; the prominent of which are pain in back, flank, and suprapubic area, hematuria, increased urinary frequency, and nocturia. These all features are not specific for GUTB and may also be found in conventional bacterial urinary tract infection.[5]

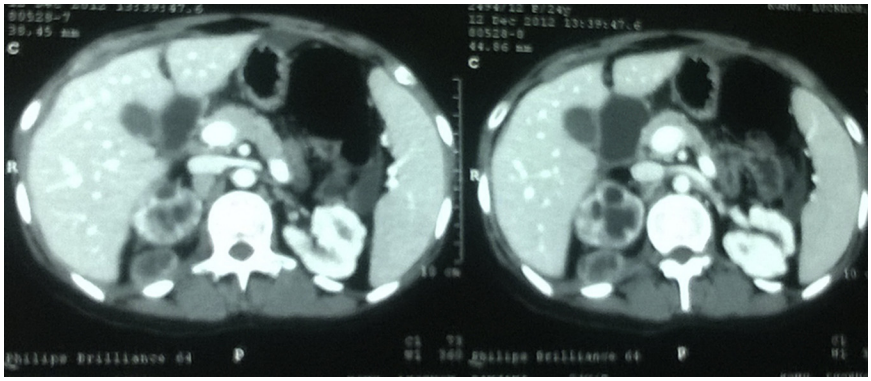

Fig. 3 – CT scan revealing right-sided hydronephrosis with collection within the calyceal system and poor renal function.

Renal TB usually occurs due to dissemination of bacilli from another primary site (most commonly the lungs). Systemic dissemination of TB is possible if infective emboli gains access to the vascular system by erosion of the wall of a vessel, usually a vein, in the primary site.[5] However, because of stringent growth requirements of the bacillus, proliferation is only possible at a limited number of sites. This includes kidney, epididymis, fallopian tube, and bone marrow. Renal medulla is the usual site of infection in renal TB.[3] The clinical consequences of an extensive renal lesion may be autonephrectomy. Ureteric involvement may also produce irregular ureteric strictures and segmental dilation, leading to obstruction and/or reflux. Recognition that ureteric obstruction and reflux sometimes may be due to TB also can prevent an unnecessary nephrectomy if active treatment including relief of obstruction is instituted early.[5]

Diagnosis is based on the clinical history, presence of AFB in urine, and growth of MTB on urine culture. CT scan is the mainstay for investigating possible GUTB and has demonstrated a positive diagnostic rate of 84.3%.[3]

Similar to TB affecting other organs, renal TB can also present in a variety of ways. This may range from simple pyuria to ESRD, including ureteric colic, epididymitis, prostatitis, interstitial nephritis, amyloidosis, and hypercalcemia in its spectrum. This leads to delay in diagnosis in a large number of cases. Appearance of calcification in a tuberculous kidney is a sign of advanced stage of the disease and is reported in up to 50% of patients with renal TB.[9] Timely diagnosis and initiation of ATT is warranted because delay in treatment can lead to irreversible organ damage and ESRD. In a country like India with high prevalence of TB and its varied presentation, when it involves the renal system, a therapeutic trial of ATT may be undertaken to avoid unnecessary surgical complications and ESRD.

According to the Revised National TB Control Programme (RNTCP) and the World Health Organization (WHO) guidelines for the treatment of extrapulmonary TB, treatment with ATT should be given for 6 months, but clinicians tend to give longer duration of treatment in such patients, which is not indicated. The caseous material and pus should be removed in every case as this shortens the duration of medical management and also prevents consequent late sequelae.[10] In patients with nonfunctioning kidneys, nephrectomy is indicated.[11]

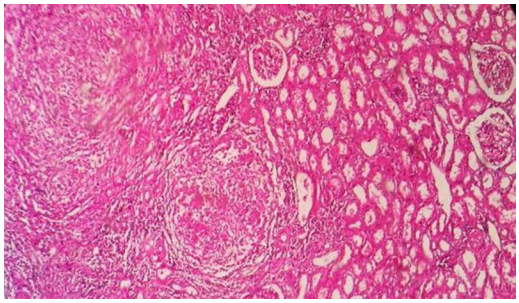

Fig. 4 – HPE of nephrectomy specimen revealing multiple well-defined granulomas in the interstitium comprising of central caseous necrosis with epitheloid cells, Langhans cells, foreign body giant cells, plasma cells, and mixed inflammatory infiltrate s/o tuberculous granulomatous inflammation.

4. Conclusion

Renal TB is an important cause of kidney disease, particularly in endemic and developing areas of the world. Delayed diagnosis and improper treatment can lead to end-stage renal dysfunction in this otherwise totally curable disease in the era of chemotherapy. Renal TB can present in wide, atypical ways and this differential should be considered in any case of pyonephrosis, particularly in endemic areas in cases where there is no response to the usual antibiotic treatment in patients of HDUN. This case highlights the importance of suspecting renal TB and prompt treatment with ATT, which may include therapeutic trail also, in any case of hydronephrosis/pyonephrosis even in the absence of signs, symptoms, or urine investigations suggestive of TB. This is particularly very important in the high prevalence endemic areas like India. Because of the varied presentations GUTB may acquire, a high level of suspicion is utmost for prompt diagnosis and early treatment. This may prevent an easily avoidable ESRD.

Conflicts of interest

The authors have none to declare.

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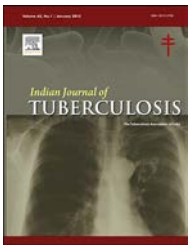

Abstracts

Xpert® MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children

Singh M, Sethi GR, Mantan M, Khanna A, Hanif M. *Int J Tuberc Lung Dis* 2016;20(6):839–843

Setting: A tertiary care teaching hospital in New Delhi, India.
Objective: To determine the sensitivity and specificity of the Xpert® MTB/RIF assay in paediatric pulmonary tuberculosis (PTB) using MGIT™ culture as gold standard.
Methods: After ethical approval had been obtained, 50 patients aged 0–14 years with suspected PTB were enrolled. Sputum/induced sputum and gastric lavage from the participants were sent for direct smear, MGIT culture and Xpert testing. Chest X-ray and tuberculin skin test (TST) were also performed. PTB diagnosis was made without considering Xpert results according to the Revised National Tuberculosis Control Programme (RNTCP) algorithm. The sensitivity and specificity of Xpert were calculated using culture as gold standard.
Results: Of 50 individuals with suspected PTB, 23 (46%) were diagnosed with PTB based on the RNTCP algorithm. Sixteen children from the PTB group (69.5%) were Xpert-positive. None in the 'not PTB' group were Xpert-positive. With culture as gold standard, Xpert sensitivity and specificity were respectively 91.6% (95%CI 59.7–99.5) and 86.8% (95%CI 71.1–95.05).
Conclusion: In almost 70% of PTB cases, a definitive diagnosis could be made within 2 h using Xpert, establishing its role as a sensitive and specific point-of-care test.

Conflicts of interest

The authors have none to declare.

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Engaging private providers and Ayurvedic practitioners in Bilaspur, India: Did it increase TB case detection?

Bhardwaj RR, Oeltmann JE, Ravichandra C, Chadda VK, Das M, Kumar AMV. *Public Health Action* 2016;6(2):154–156

To find 'missing' tuberculosis (TB) cases, in November 2014 we trained private practitioners (PPs) and Ayurvedic practitioners (APs; Indian system of medicine) in Bilaspur district, India, to identify patients with presumptive TB and refer them to sputum microscopy centres. To reinforce this training, we sent weekly text message reminders during January–March 2015. All 50 APs and 23 of 29 PPs participated. The number of patients with presumptive TB referred by the PPs and APs increased from 38 (January–March 2014) to 104 (January–March 2015), and the number of smear-positive TB patients diagnosed increased from 5 to 16, a 220% increase. While the intervention increased the number of referrals, it did not impact case detection at district level, due to the short duration of the intervention and the non-dominant private sector.

Conflicts of interest

The authors have none to declare.

http://dx.doi.org/10.1016/j.ijtb.2016.09.003

Isoniazid hair concentrations in children with tuberculosis: A proof of concept study

Mave V, Chandanwale A, Kinikar A, et al. *Int J Tuberc Lung Dis* 2016;20(6):844–847

Assessing treatment adherence and quantifying exposure to anti-tuberculosis drugs among children is challenging. We undertook a 'proof of concept' study to assess the drug concentrations of isoniazid (INH) in hair as a therapeutic drug monitoring tool. Children aged <12 years initiated on a thrice-weekly treatment regimen including INH (10 mg/kg) for newly diagnosed tuberculosis were enrolled. INH concentrations in hair were measured using liquid chromatography-tandem mass spectrometry at 1, 2, 4 and 6 months after initiating anti-tuberculosis treatment. We found that INH hair concentrations in all children on thrice-weekly INH were detectable and displayed variability across a dynamic range.

Conflicts of interest

The authors have none to declare.

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Loop-mediated isothermal amplification (LAMP) assay for speedy diagnosis of tubercular lymphadenitis: The multi-targeted 60-min approach

Sharma M, Sharma K, Sharma A, Gupta N, Rajwanshi A. *Tuberculosis* 2016;99

Introduction: Tuberculous lymphadenitis (TBLA), the most common presentation of tuberculosis, poses a significant diagnostic challenge in the developing countries. Timely, accurate and cost-effective diagnosis can decrease the high morbidity associated with TBLA especially in resource-poor high-endemic regions. The loop-mediated isothermal amplification assay (LAMP), using two targets, was evaluated for the diagnosis of TBLA.
Material and methods: LAMP assay using 3 sets of primers (each for IS6110 and MPB64) was performed on 170 fine needle aspiration samples (85 confirmed, 35 suspected, 50 control cases of TBLA). Results were compared against IS6110 PCR, cytology, culture and smear.
Results: The overall sensitivity and specificity of LAMP assay, using multi-targeted approach, was 90% and 100% respectively in diagnosing TBLA. The sensitivity of multi-targeted LAMP, only MPB64 LAMP, only IS6110 LAMP and IS6110 PCR was 91.7%, 89.4%, 84.7% and 75.2%, respectively among confirmed cases and 85.7%, 77.1%, 68.5% and 60%, respectively among suspected cases of TBLA. Additional 12/120 (10%) cases were detected using multi-targeted method.
Discussion: The multi-targeted LAMP, with its speedy and reliable results, is a potential diagnostic test for TBLA in low-resource countries.

Conflicts of interest

The authors have none to declare.

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Lipoarabinomannan-responsive polycytotoxic T cells are associated with protection in human tuberculosis

Busch M, Herzmann C, Kallert S, et al. *Am J Respir Crit Care Med* 2016;194(3):345–355
Rationale: The development of host-targeted, prophylactic, and therapeutic interventions against tuberculosis requires a better understanding of the immune mechanisms that determine the outcome of infection with *Mycobacterium tuberculosis*.
Objectives: To identify T-cell-dependent mechanisms that are protective in tuberculosis.
Methods: Multicolor flow cytometry, cell sorting and growth inhibition assays were employed to compare the frequency, phenotype and function of T lymphocytes from bronchoalveolar lavage or the peripheral blood.
Measurements and main results: At two independent study sites, bronchoalveolar lavage cells from donors with latent tuberculosis infection limited the growth of virulent *Mycobacterium tuberculosis* more efficiently than those in patients who developed disease. Unconventional, glycolipid-responsive T cells contributed to reduced mycobacterial growth because antibodies to CD1b inhibited this effect by 55%. Lipoarabinomannan was the most potent mycobacterial lipid antigen (activation of 1.3% T lymphocytes) and activated CD1b-restricted T cells that limited bacterial growth. A subset of IFN-γ-producing lipoarabinomannan-responsive T cells coexpressed the cytotoxic molecules perforin, granulysin, and granzyme B, which we termed *polycytotoxic T cells*. Taking advantage of two well-defined cohorts of subjects latently infected with *Mycobacterium tuberculosis* or patients who developed active disease after infection, we found a correlation between the frequency of polycytotoxic T cells and the ability to control infection (latent tuberculosis infection, 62%; post-tuberculosis patients, 26%).
Conclusions: Our data define an unconventional $CD8^+$ T-cell subset (polycytotoxic T cells) that is based on antigen recognition and function. The results link clinical and mechanistic evidence that glycolipid-responsive, polycytotoxic T cells contribute to protection against tuberculosis.

Conflicts of interest

The authors have none to declare.

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Trend in the incidence of smear-positive tuberculosis in a district in South India after DOTS implementation

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Setting: One baseline and three repeat surveys of the prevalence of tuberculosis (TB) disease were conducted in 1999–2008 in rural South India, where the DOTS strategy was implemented in 1999. The impact of DOTS on prevalence was documented, but not its impact on incidence.
Objective: To ascertain epidemiological trends in the incidence of smear-positive TB.
Design: All persons aged □15 years (range 83,000–92,000) were examined using chest radiography (CXR); chest symptoms and history of anti-tuberculosis chemotherapy were noted in all four surveys. Sputum was collected from eligible participants and tested using direct smear and culture, and for drug susceptibility. As follow-up surveys were not frequent, survey cases and cases directly notified under DOTS were combined to estimate the incidence of smear-positive TB.
Results: Coverage was consistently high in all the repeat surveys, at □80% for CXR and symptom recording, and at □95% for sputum examination. The annual incidence of smear-positive TB was respectively 112, 80 and 76 per 100,000 population in 2001–2003, 2004–2006 and 2006–2008. The overall decline observed was 7.5% per annum.
Conclusion: A well-implemented DOTS strategy can lead to a reduction in the TB burden in the community.

Conflicts of interest

The authors have none to declare.

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