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Research Article

Clinico-pathological profile, radiological presentation and drug susceptibility pattern of new smear positive (category I) pulmonary tuberculosis: a single centre experience in Delhi, India

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ABSTRACT

Background: Aim of current study was to determine the clinical characteristics, radiological, laboratory features and anti-tubercular drug sensitivity in new smear positive (category I) pulmonary tuberculosis cases in a tertiary care dedicated TB OPD, Delhi.
Methods: The study was a cross-sectional observational study and consists of 100 cases of new smear positive pulmonary tuberculosis cases (category I) irrespective of age and sex. The sputum were collected, stained with Ziehl-Nielsen (Z-N) staining and ultimately inoculated on Lowenstein-Jensen (L-J) media for six weeks. All sputum smear positive cases were subjected to culture and drug-susceptibility testing by 1% proportion method on Lowenstein-Jensen (LJ) medium. The Drug-Susceptibility Testing (DST) for isoniazid (INH), rifampicin (R-cin), ethambutol (EMB) and streptomycin (SM) were performed.
Results: The age & sex distribution of 100 patients showed that majority of the patients (79%) belonged to 2nd, 3rd and 4th decades & 60 % were males and 40% were female with male to female ratio 3:2 respectively. Cough (83%), fever (77%) and weight loss (76%) were the most common presenting clinical features. The chest X-ray of 100 smear positive patients showed that 53% of patients had evidence of 35% unilateral and 18% bilateral consolidation and 46% had cavitary lesions on chest X-ray (PA view) with 37% and 9% of patients having unilateral and bilateral cavities respectively. Of these 82 culture positives, 56.1% (n=46) were susceptible to all first-line anti-tubercular drugs, while 43.9% (n=36) were resistant to mostly one or other anti-tubercular drugs (INH, R-cin, SM or EMB).
Conclusion: We stressed the importance of early diagnosis of new cases by clinico-pathological features, identifying of drug resistance trends in anti-tubercular treatment naïve patients, in order to assess the efficacy of current interventions. Overall, these findings emphasize the importance of early diagnosis of drug resistance pattern of M. tuberculosis isolates to anti-tubercular in category I patients as well as its association with HIV across the country to timely modify and strengthen the national programs in order to prevent the emergence of MDR-TB strains and avert the threat of XDR-TB.

Keywords: Pulmonary tuberculosis, New case, Category I, Drug susceptibility, Drug-resistance

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by a bacterium, Mycobacterium tuberculosis. It spread through the air by a person suffering from TB. It is a global pandemic disease. All TB control programs were not successful due to the emergence of multidrug resistance in M. tuberculosis strains. It remains a major global health problem including India. Early diagnosis of new cases on the basis of clinical features, sputum and radiological examination is required for early initiation of anti-tubercular therapy.

Some of the new cases may be resistant to one or more first-line anti-tubercular drugs. So incorporation of Drug Sensitivity/Susceptibility Testing (DST) may be very useful to avoid further development of Multi-Drug Resistance (MDR) and treatment failure. As Multi-Drug Resistant (MDR) and Extensively-Drug Resistant (XDR) tuberculosis (TB) are very serious threat to the national TB control programs of the developing countries, and the situation is further worsened by the Human Immunodeficiency Virus (HIV) co-infection.

WHO recognized the importance of the situation and trends of MDR and XDR strains of Mycobacterium tuberculosis as barriers to the achievement of the WHO's Global Plan's objectives by 2015.

Burden of disease

In 2012, an estimated 8.6 million people developed TB and 1.3 million died from the disease (including 320000 deaths among HIV-positive people). Tuberculosis (TB) continues to be a major public health problem in India, with an estimated 2.3 million new cases annually, making it the highest TB burden country in the world. In 2010, India alone accounted for an estimated one quarter (26%) of all TB cases worldwide.[1]

The prevalence of TB disease is an important epidemiological index to measure the burden in a community and if measured periodically, will enable trends in disease prevalence to be observed over time. In 2012, case detection rate of new smear positive TB cases was 68% with a treatment success rate of 88%. Globally in 2012, an estimated 450000 people developed MDR-TB and there were an estimated 170000 deaths from MDR-TB.[1]

Definitions of tuberculosis cases

Smear-positive pulmonary case[2]

A patient with one or more initial sputum smear examinations (direct smear microscopy) AFB+ve or one sputum examination AFB+ve and radiographic abnormalities consistent with active pulmonary tuberculosis as determined by a clinician

New case[2]

A patient who has never had treatment for tuberculosis or who has taken anti-tubercular drugs for less than one month.

Drug resistance[3]

Drug resistance in mycobacteria is defined as a decrease in sensitivity to a sufficient degree to be reasonably certain that the strain concerned is different from a sample of wild strains of human type that have never come in contact with the drugs.

Types of drug resistance

Drug resistance in TB may be broadly classified as primary or acquired. When drug resistance is demonstrated in a patient who has never received anti-tubercular treatment previously, it is termed primary resistance. Acquired resistance is that which occurs as a result of specific previous treatment. The level of primary resistance in the community is considered to reflect the efficacy of control measures in the past, while the level of acquired resistance is a measure of on-going TB control measures. However, the World Health Organization (WHO) and the International Union against Tuberculosis and Lung Diseases (IUATLD), after lot of discussions, have replaced the term primary resistance by the term "drug resistance among new cases" and acquired resistance by the term "drug resistance among previously treated cases".[3,4]

The present study was a keen effort to find out the trend in clinical features, microbiological and radiological presentation and anti-tubercular drug susceptibility patterns of category I pulmonary tuberculosis in patients attending a tertiary care dedicated TB OPD, Delhi.

METHODS

Total randomly selected 100 patients of newly diagnosed pulmonary tuberculosis with or without glandular involvement attending TB Out-patient Department of a tertiary care hospital over a period of 6 months were included in the study. The patients were selected irrespective of age or sex with symptoms such as cough, low grade fever, hemoptysis, chest pain, weight loss, loss of appetite or anorexia and glandular swelling for at least 3 weeks. A detailed history of previous anti-tubercular treatment was taken and only those patients who had never had treatment for tuberculosis (new case or category I) were included. Ethics permission was obtained and written informed consent was sought before recruiting patients for the observational study. A detailed history was taken with particular reference to demographic information, socioeconomic status, Bacillus Calmette-Guerin (BCG) vaccination and family history of tuberculosis. A thorough general physical and systemic examination was carried out. All cases were subjected to

hemogram, urine-routine/microscopy, hepatic and renal function tests, blood sugar, Mantoux test, Enzyme Linked Immune-Sorbent Assay (ELISA) for Human Immunodeficiency Virus (HIV), and chest X-ray (PA view). All patients were directed to collect the early morning sputum specimen in a sterilized wide-mouthed bottle with a tightly fitting cork stopper. Sputum was sent for smear for Acid-Fast Bacilli (AFB) and culture/sensitivity, on three consecutive days. All sputum samples were transported to the microbiology lab as soon as possible after collection. The smears were screened for Acid-Fast Bacilli (AFB) and positive smears were graded as per Revised National Tuberculosis Control Programme (RNTCP) guidelines.[5]

Screening for Human Immunodeficiency Virus (HIV) infection was done in all the patients after consent. FNAC of glandular lesions and other radiological investigations were done in relevant cases. Early morning sputum samples were collected from each patient for three consecutive days. The sputum was first concentrated by Petroff's method.[6] After concentration, a smear was made on a slide and smear examination done using Ziehl-Neelsen staining technique.[7] Culture for isolation of Mycobacterium tuberculosis was done on Lowenstein-Jensen (L-J) medium. Cultures were examined for growth after incubation at 37°C for 4 days (for rapidly growing mycobacteria and contaminants) and every week thereafter up to 8 weeks. The colonies of Mycobacterium tuberculosis were defined as rough, crumbly, waxy, non-pigmented (buff colored). Negative cultures were defined as no growth after 8 weeks. The positive cultures showing AFB were identified as M. tuberculosis based on the results of growth on LJ medium containing p-nitrobenzoic acid and niacin tests.[8,9] Drug susceptibility testing of patient's sputum samples was done using Proportion method.[10] Only one culture positive sputum sample from each patient was selected for drug susceptibility testing to isoniazid, rifampicin, ethambutol and streptomycin. The numbers of colony forming units growing on the medium containing the drug were compared with the number on the control plate. The proportion of resistant cells in the total viable population of the original inoculums were then calculated and expressed as percentage. Significant proportion of growth above which the isolate was labeled resistant was set at 1% as per the recommendations.

RESULTS

In the present observational study, 100 cases of newly diagnosed sputum positive tuberculosis, with no prior history of anti-tubercular chemotherapy treatment (category I) presenting first time at the clinic were selected.

The mean age of tuberculosis patients was 30.51 ± 17 years. The age range of the patients included in the study was 11-76 years (Table 1).

Table 1: Demographic composition of the tuberculosis patients included in the study.

	Male	Female	Total
Age (years)			
11-20 years	7	12	19
21-30 years	23	20	43
31-40 years	12	5	17
41-50 years	8	1	9
51-60 years	6	3	9
61-70 years	2	0	2
>71 years	1	0	1
Total	60	40	100
Education			
Literate	56	37	93
Illiterate	04	03	07
Occupation			
Unemployed	11	07	18
Household work	03	27	30
Retired	04	02	06
Laborer	18	15	33
Service	11	04	15
Skilled worker	08	02	10
Student	05	03	08
Marital status			
Never married	26	16	42
Married	32	21	53
Widowed	02	03	05

Table 2: Symptoms of included (Category I) tuberculosis patients (n=100).

Symptom	Number	%
Cough	83	83%
Fever	77	77%
Haemoptysis	15	15%
Chest pain	48	48%
Glandular swelling	3	3%
Weight loss	76	76%
Loss of appetite	73	73%

The average duration of symptoms in most patients varied from 12-30 days.

Table 3: Tuberculin testing (Mx) results in category I patients (n=100).

Response	Number	%
Positive (reactors)	89	89%
Negative (non-reactors)	8	8%
Borderline	3	3%

Mantoux test (Mx) was done on each patients with 1 Tuberculin Unit of PPDRT 23 with Tween 80.[11] Mx test detects delayed hypersensitivity to tuberculin. The test demonstrates infection due to tuberculosis on the basis of

the skin indurations at the site of injection and was read after 48-72 hours of intra-dermal injection.

All the hundred patients underwent X-ray chest (PA view) examination and extent of radiological lesion was classified as minimal, moderately advanced and far advanced, as per American thoracic society classification. A standard 6-foot posterioanterior (PA) chest radiograph was obtained for all patients.

Cavitary disease was defined as the presence of a gas-containing lucent space at least 1 cm in diameter within the lung parenchyma surrounded by an infiltrate or fibrotic wall greater than 1 mm thick. A limitation of our study was the presence of cavitary disease was made using standard PA chest radiographs. Small cavitary lesions may have been found in some of the non-cavitary patients if computed tomography of the chest had been used.

Table 4: Radiological extent of the disease in all patients included in the study (n=100).

Extent	Number	%
Minimal lesion	43	43%
Moderately	54	54%
Far advance lesion	3	3%

Table 5: Radiological features of patients included in the study (n=100).

Radiological feature	Number	%
Consolidation	53	53%
• Unilateral	35	35%
• Bilateral	18%	18%
Cavity	46	46%
• Unilateral	37	37%
• Bilateral	9	9%
Others	2	2%

Table 6: Sputum status of category I patients before the start of anti-tubercular chemotherapy (n=100).

	Negative (%)	Positive (%)			
		1+	2+	3+	Total
Direct smear AFB	-	44	37	19	100
Culture	18	82			

Table 7: Overall drug susceptibility in initial culture positive cases (n=82).

	Culture positives (n=82)	
	No.	%
Drug susceptible	46	(56.1%)
Drug resistant	36	(43.9%)

Table 8: Detailed data of drug susceptibility profile of category I patients (n=82).

Resistance to	Numbers	%
INH alone	11	13.4
R-cin alone	2	2.4
SM alone	3	3.7
EMB alone	3	3.7
INH+ SM/EMB	6	7.3
R-cin + SM/EMB	-	-
INH + R-cin ± SM/EMB	11	13.4

Table 9: Trends on individual drug resistance (primary) to M. tuberculosis in India (1999-2010).[12]

Location	Year	Resistance to single drug			
		INH	SM	R-cin	EMB
North Arcot (Pondicherry)	1989-90	13.0	4.0	0.07	ND*
North Arcot (Pondicherry)	1989-98	6.0	4.0	0.2	ND
Jaipur	1988-91	7.6	5.2	1.9	2.0
New Delhi	1990-91	18.5	-	1.9	-
Military hospital, Pune	1992-93	3.2	8.2	4.0	-
Tamil Nadu state	1997	15.4	6.8	4.4	-
Raichur	1999	18.7	7.2	2.5	-
Wardha	2000	15.0	7.6	0.5	-
Jabalpur	2002	16.5	7.0	1.8	-
Present study	2009-10	13.4	3.7	2.4	3.7

*ND - not done

Table 10: Trends on multi-drug resistance (primary) to M. tuberculosis in India (1999-2010).[13]

District (Zone)	Intake period	No. of patients	Primary multi-drug resistance (%)
North Arcot (South)	1999	282	2.8
Raichur (South)	1999-2000	278	2.5
Wardha (West)	2000-2001	197	0.5
Jabalpur (West)	2001-2002	273	1.0
Hoogly (East)	2000-2001	350	3.0
Mayurbanj (East)	2000-2002	343	0.7
Present study (Delhi)	2009-10	100	13.4%

DISCUSSION

The aim of this was to study clinic-pathological, radiological presentation and prevalence of initial drug resistance in 100 smear positive new cases (category I) attending the TB Clinic of a tertiary care hospital. All patients tested were sputum smear positive for AFB and percentage grading as +3, +2, +1, and scanty were 19%, 37% and 44%, respectively. Of all, 97 patients had pulmonary tuberculosis and 3 patients had

lymphoglandular tuberculosis in addition to pulmonary involvement. Of the included 100 patients, 89% were tuberculin skin test (TST) positive (reactors) with induration >10 mm, 8% were Mx negative (non-reactors) i.e. induration <10 mm and 3% were borderline reactors with induration of 10 mm.

Based on CDC guidelines for the classification of TST reactions, the TST result was divided into categories of 0-4 mm, 5-9 mm, 10-14 mm, and ≥15 mm. A TST result of 0-4 mm was considered negative and a result ≥5 mm was considered positive. A positive TST result, consisting of measurable skin induration after the injection of tuberculin purified protein derivative, is part of a delayed-type hypersensitivity response of the host immune system memory T cells sensitized by prior mycobacterial exposure.[14]

53% of patients had evidence of consolidation with 35% having unilateral and 18% having bilateral consolidation. 46% had cavitatory lesions on chest X-ray (PA view) with 37% and 9% of patients having unilateral and bilateral cavities respectively.

Of the selected hundred 100 patients, all were direct smear positive from AFB, with 44% having 1+, 37% having 2+ and 19% of the patients having 3+ sputum positivity. In developing countries, sputum Acid-Fast Bacilli (AFB) smear microscopy is the primary tool for detecting pulmonary tuberculosis. The Ziehl-Neelsen (ZN) method is commonly used for staining sputum smears because of its simplicity and low cost. Culture positivity was seen in 82% cases; while 18% were smear positive, but culture negative.

Smear-positive/culture-negative results are indeed a challenge to interpret and manage, especially in the absence of clinico-radiographic and mycobacteriologic history regarding the patients. Nevertheless, possible explanations may be continued expectoration of dead organisms or non-cultivable non-TB mycobacteria unable to proliferate in the environment of a standard AFB culture or excessive delay (e.g., 5 days) between specimen collection and culture inoculation, lowering the sensitivity of culture.

Of these 82 culture positives, 56.1% (n=46) were susceptible to all first-line anti-tubercular drugs, while 43.9% (n=36) were resistant to mostly one or other anti-tubercular drugs (INH, R-cin, SM or EMB).

There are two ways that people get drug resistant TB. Firstly, people get acquired drug resistant TB when their TB treatment is inadequate. This can be for a number of reasons, including the fact that patients fail to keep to proper TB treatment regimes, the wrong TB drugs are prescribed, or sub-standard TB drugs are used for treatment. Secondly, transmitted or primary drug resistant TB results from the direct transmission of drug resistant TB from one person to another. The occurrence and prevention of primary drug resistant TB has largely been neglected during the development of global TB control programs.

Resistance was more common with INH alone (13.4%). Drug resistance to INH and others (SM or EMB) was 7.3% (n=6) and none in the R-cin group. The total number of Multi-Drug Resistance (MDR) cases i.e. resistance to INH, R-cin and/or others (SM/EMB) was 20.7% (n=17). Of all, only 2 cases (2%) were diagnosed as HIV+ve and they have shown resistance to one or more anti-tubercular drugs, which confirms the finding of other previous study conducted on patients with TB.[15,16]

One study from India among 271 new smear and culture positive patients (category I), was observed that initial drug resistance to any drug was 27% with the MDR pattern being 2.2%.[15]

Globally in 2012, data from drug resistance surveys and continuous surveillance among notified TB cases suggest that 3.6% of newly diagnosed TB cases and 20% of those previously treated for TB had MDR-TB. The highest levels of MDR-TB are found in Eastern Europe and Central Asia, where in some countries more than 20% of new TB cases and more than 50% of those previously treated for TB have MDR-TB.[1] Looking at the above table 9 that individual primary drug resistance to first-line anti-tubercular drugs studied has varied from region to region and year to year.

Observations from reliable accredited mycobacteriology laboratories from India suggest that the prevalence of MDR-TB is quite low in new TB cases (<3%) compared with our study patients (13.4%) (Table 10). Drug-resistant TB has microbial, clinical, and programmatic causes. From a microbiological perspective, the resistance is caused by a genetic mutation that makes a drug ineffective against the mutant bacilli. An inadequate or poorly administered treatment regimen allows drug-resistant mutants to become the dominant strain in a patient infected with TB.[17]

Our study has several limitations. First, we studied only patients with documented smear positive new case who were anti-tubercular treatment naive. We have excluded those patients or new cases, whom were on anti-tubercular treatment less than one month though they fits into new case (category I) definitions. Second, it was a cross-sectional observational study, so we cannot predict secondary drug resistance case and treatment outcomes from our study results.

CONCLUSION

The emergence of resistance to drugs used to treat tuberculosis (TB), and particularly Multi-Drug-Resistant TB (MDR-TB), has become a significant public health problem in a number of countries including India and an obstacle to effective TB control.

This study showed that 13.4% cases were resistant to more than two first line anti-tubercular drugs being declared as having MDR-TB bacilli, who need treatment with second line anti-TB drugs for treatment initiation. This study also revealed that INH resistance is seen in 13.4% cases in this geographical area. Hence, there is a need to start MDR-TB regimen in cases of drug resistant TB.

The classification of drug resistance as primary or acquired is used as an indicator of the efficiency of national tuberculosis programme and in the adjustment and development of these programmes. The rate of primary drug resistance is interpreted as an epidemiological indicator for long-term surveillance of the quality of tuberculosis treatment in the community. The rate of acquired drug resistance reflects the efficacy of management of individual patients.

A high index of suspension on the basis of clinical features and prompt investigations including drug susceptibility testing in new sputum positive PTB cases and early initiation of treatment will ensure decreasing development the MDR & XDR-TB and TB related morbidity and mortality. In conclusion, the present study highlights drug susceptibility tests should be done for patients to all new cases (Category I) for early identification of drug resistance.

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