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Editorial

CAN NEWER DIAGNOSTIC MICROBIOLOGICAL ASSAYS GUIDE EARLY TUBERCULOSIS MANAGEMENT?

[*Indian J Tuberc 2011; 58:51-53*]

Tuberculosis remains the single infectious disease causing the highest mortality in humans, leading to three million deaths annually - about five deaths every minute. In India, TB kills two persons every three minutes - nearly 1,000 every day. The definitive detection, identification and susceptibility testing of *Mycobacterium tuberculosis* is therefore vital for clinicians to confirm the diagnosis of tuberculosis and then to plan the appropriate management protocol for affected patients.

The role of the microbiology laboratory in the diagnosis and management of tuberculosis involves the rapid detection and isolation of mycobacteria, the identification of the mycobacterial species or complex isolated, and the determination of susceptibilities of the organisms to anti-TB drugs. The challenge now is to combat the increasing HIV co-infection and Multi-drug Resistant Tuberculosis (MDR-TB). It is estimated that 500,000 new cases of MDR-TB occurred in the world in 2007.[1] Extensively Drug-resistant TB (XDR-TB) is an emerging threat and to reduce the resulting morbidity and mortality, rapid diagnostic tests are the need of the day not only for better patient care but for the national TB control programme as well.

The time-tested method used for years was the traditional culture of *M. tuberculosis* on conventional solid media. Anti-mycobacterial susceptibility takes an additional length of time, though not as long as the initial culture. These lengthy processes require several weeks for a confirmatory result. The newer methods shall be useful in reducing the time lag between the presentation and initiation of specific anti-mycobacterial treatment, and need to be not only rapid but reliable as well.

In recent years, the use of liquid culture media in conjunction with automated TB culture systems has significantly shortened the culture time needed for the detection of mycobacteria, both tuberculous and non-tuberculous types. This method is more sensitive and can detect more cases than the conventional TB culture method. Not only are errors minimized, but also sophisticated algorithms in these computerized TB culture systems detect the presence or absence of mycobacteria, much faster – cutting down the time taken for getting the culture result from six-eight weeks of the conventional TB culture to a more convenient two-four weeks.

Microscopic observation broth-drug susceptibility assay (MODS) is an 'in-house' liquid culture method that relies on microscopic observation of serpentine cording, characteristic of *M. tuberculosis* growth that gives uniformly good results, particularly for rifampicin (RMP) and isoniazid (INH). MODS is also a rapid and sensitive alternative method for the isolation of *M. tuberculosis* from children.[2]

Other recent methods include several phage-based assays, thin-layer agar microcolony method, calorimetric tests that also are fairly rapid and simple, but are not yet part of the routine diagnostics regimen.

Delineation of the specific genes of *M. tuberculosis* has led to the development of molecular tests for tuberculosis. The detection of mycobacteria at the molecular level has revolutionized TB diagnostics,

reducing the turn around time from weeks to days. All molecular assays start with extraction and amplification of the target gene from the sample.

A PCR (Polymerase Chain Reaction) on a sputum (or other samples) for TB bacilli can be performed in one day with a sensitivity of 60-100%. Additionally, this method also allows direct determination of rifampicin resistance (by detecting the rpoB gene) that is particularly important because it is a marker of MDR-TB and thus, a strong predictor of treatment outcome. Using PCR tests in conjunction with culture for extra-pulmonary tuberculosis also have been found to be 100% specific and having 100% positive predictive value for the early detection of *M. tuberculosis.*

Genotyping or DNA fingerprinting determines the clonal origin of culture isolates and can be used to trace the spread of TB bacteria. This method is also useful in investigating outbreaks of tuberculosis, in determining whether new episodes of tuberculosis are due to reinfection or reactivation, confirming laboratory cross-contamination, and determining patterns of *M. tuberculosis* transmission within communities.

Progress in understanding of human host responses has helped develop the most recent, as well as controversial, addition to the TB tests - the Interferon Gamma Release Assays (IGRAs). These are superior, in comparison to the Mantoux test, for detecting confirmed active TB disease, but have limited value in many developing countries where TB is endemic and majority of the population has been exposed to the infection earlier.[3] The advantages of IGRAs over the Mantoux test include – a single visit to the laboratory, being more objective (reducing operator-dependent variable interpretation) and for diagnosing Latent Tuberculosis Infection (LTBI), as well as detecting active TB patients.[4] These tests can provide significant advantage in diagnosis of TB in HIV infected patients as well as young children. The test can determine if a positive Mantoux result in a child who received a BCG vaccine is from the BCG (negative IGRA) or from LTBI (positive IGRA).[5] However, it must be noted that most patients with latent TB infection remain positive after treatment and these tests should not be used as a marker to monitor the effect of therapy.

The alarming increase in MDR-TB and the emergence of XDR-TB, potential institutional transmission, and the rapid mortality of MDR-TB and XDR-TB patients with HIV co-infection, have highlighted the urgency for rapid screening. Their presence poses a formidable challenge to TB control due to its complex diagnostic and treatment challenges. The annual global MDR-TB burden is estimated at around 490, 000 cases, or 5% of the global TB burden; however, less than 5% of existing MDR-TB patients are currently being diagnosed as a result of serious laboratory capacity constraints.[6] Early identification of MDR-TB allows much faster investigation, diagnosis and treatment of MDR and XDR-TB. A newer diagnostic system called as Line Probe Assay (LPA) that is a molecular based technology, has the efficiency to detect MDR in a couple of hours. The WHO has recommended the use of LPAs for rapid screening of MDR-TB in low and middle income settings. LPAs use multiplex PCR amplification and reverse hybridization to identify *M. tuberculosis* complex and mutations to genes associated with rifampicin and isoniazid resistance. LPA can be performed directly from Acid Fast Bacilli (AFB) smear-positive sputum, or from culture isolates, and provide results in one-two days.[7] A recent systematic review concluded that LPAs are highly sensitive and specific for detection of rifampicin resistance ($\geq$ 97% and $\geq$ 99%) and isoniazid resistance ($\geq$ 90% and $\geq$ 99%) on culture isolates and smear-positive sputum, and conventional drug susceptibility testing for detection of MDR-TB was 99%.[8]

The GeneXpert Method is a test that can simultaneously identify *M. tuberculosis* and resistance to rifampicin. A recent study has shown a sensitivity of over 97% and specificity of the Xpert assay for pulmonary specimens the usefulness of the Xpert assay applied to the rapid diagnosis of extrapulmonary tuberculosis.[9]

Thus, microbiological tests are essential for management of TB because they are the critical step in confirming the diagnosis. Faster turnaround times available with the newer assays are invaluable for early and better patient management. The cost-effectiveness of some of these newer diagnostic microbiological assays has ensured their adoption in the diagnosis of tuberculosis by the Revised National TB Control Programme.

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Original Article

PREVALENCE OF XDR TB CASES – A RETROSPECTIVE STUDY FROM A TERTIARY CARE TB HOSPITAL

V.P. Myneedu, P. Visalakshi, A.K. Verma, D. Behera and M. Bhalla

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Summary

Background: The emergence of XDR –TB strains is a major roadblock in the successful implementation of TB control programmes. This further leads to high morbidity and mortality, especially in immuno-compromised patients. Identification and observation of resistance patterns of XDR-TB strains may help clinicians manage MDR-TB cases, the treatment line of which is expensive, time-taking and involves intake of toxic drugs with many side-effects. Our study is aimed to find out the prevalence of XDR-TB among the MDR-TB strains isolated in a tertiary care hospital.

Material & Methods: The study population consisted of 223 patients of tuberculosis who were culture positive and *Mycobacterium tuberculosis* was resistant to Rifampicin and Isoniazid during January 2007 to December 2009. Each patient had submitted two sputum samples i.e. spot and morning. The identified *Mycobacterium tuberculosis* complex was subjected to drug sensitivity testing by first and second line drugs by proportion and absolute concentration methods as per standard procedure .

Results : The results showed that 20.17% strains (45/223) were XDR-TB strains. Most of these strains showed resistance to four drug combination viz. KM, ETH, OFX & PAS (5.82%), KM & OFX (3.13%), OFX, KM and ETH (1.79%), 1.34% strains showed resistance to all the drugs i.e. pan resistance and other combinations in the remaining strains. Nearly 80% of the XDR-TB strains showed resistance to three or more drugs combination pattern.

Conclusion: The multidrug resistant TB cases need urgent and timely sensitivity report for second line ATT drugs to help clinicians start proper drug combinations to treat MDR-TB patients. ***[Indian J Tuberc 2011; 58:54-59]***

Key words: *Mycobacterium tuberculosis*, Multi Drug Resistant TB (MDR-TB), Extensively Drug Resistant TB (XDR-TB), Drug sensitivity testing, Proportion method

INTRODUCTION

Tuberculosis, a well-known bacterial disease for the last 5000 years, is still infecting nearly one-third of world population with a daily addition of 5000 new cases and loss of two lives every third minute[1]. In India, 1.9 million new cases are reported every year, of which 0.8 millions are 'infectious smear positive TB cases'. According to WHO, death rate due to TB in India is nearly 28 per 1,00,000 population, which is the highest death rate among all other communicable diseases and accounts for 26 per cent of all avoidable adult deaths[2].

The previous studies in India showed that three per cent of MDR-TB is seen in new tuberculosis cases and 17.2 per cent among retreatment cases. Ramachandran *et al* had reported 3.2% of XDR strains among the MDR isolates in a field study from Gujarat[3-5]. Information related to MDR-TB is showing relatively less number of cases but what is frightening is the number of XDR-TB cases, which is really a potential threat to healthy population of India.

In countries like India, most clinicians are not adhering to the antibiotics policy and are rampantly using second line drug treatment even in the absence of sensitivity reports. Such a malpractice may culminate in the outbreak of TB and newer TB strains, viz. XDR-TB strain, etc., beyond control. The emergence of XDR-TB strains is threatening the commitment underlying the DOTS Plus programme that intends to provide high quality service in diagnosis and treatment of MDR-TB.

XDR-TB is defined as strain of *Mycobacterium tuberculosis* resistant to isoniazid,

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rifampicin, one of the fluoroquinolones and any one of three injectible drugs, i.e. kanamycin, amikacin and capreomycin[6,7]. The drug sensitivity test data showed that XDR strains are prevalent all over the world except Antarctica and many more reports are coming in recent years[8]. According to available second line antituberculous drug sensitivity reports, the prevalence of XDR-TB cases amongst the MDR-TB varies from 5% - 15% around the world [9.10]. There is paucity of reports showing XDR-TB cases from India. The DST report mostly by non-accredited laboratories from India showed 5-16 per cent XDR-TB strains among MDR-TB strains[11]. Our accredited laboratory is carrying out DST for first line ATT drugs for the last 10 years and second line ATT drugs for the last three years.

This study is an attempt to find out the true prevalence of XDR-TB cases among the MDR-TB patients.

MATERIAL AND METHODS

It is a retrospective study conducted by Department of Microbiology of Lala Ram Swarup Institute of TB and Respiratory Diseases in New Delhi. The study population consisted of 223 patients of tuberculosis who were culture positive and *Mycobacterium tuberculosis* was resistant to rifampicin and isoniazid during January 2007 to December 2009. Each patient had submitted two sputum samples i.e. spot and morning according to the RNTCP guidelines. The culture was done on Lowenstein-Jensen (LJ) media following decontamination and concentration by modified Petroff's method[12]. The positive cultures were identified by niacin test, catalase test and sensitivity to PNB test. The identified *Mycobacterium tuberculosis* complex was subjected to drug sensitivity testing by first line drugs viz. isoniazid (H),rifampicin (R), pyrazinamide (Z), ethambutol (E) and second line drugs, viz. kanamycin (KM), ethionamide (ETH), capreomycin (CPM), amikacin (AK), ofloxacin (OFX), para aminosalycylic acid (PAS), and cycloserine (CYS)] by proportion method and absolute concentration method as per standard procedure[13].

RESULTS

During the period January, 2007 to December, 2009, the randomly selected 223 MDR-TB strains were subjected to second line anti-tuberculous drugs sensitivity testing. The 223 MDR-TB isolates were tested with kanamycin, ethionamide, capreomycin, amikacin, ofloxacin, PAS, and cycloserine, which were used in the treatment of MDR-TB patients. During the sensitivity testing, 28 different resistance patterns were observed amongst the aforementioned drugs. The overall highest number of resistant strains were seen with ofloxacin (69%) followed by resistance to ethionamide (39%), PAS (27%), kanamycin (20%), capreomycin (10%), amikacin (4%) and cycloserine (3%) (Table-1).

As per WHO definition of XDR-TB[6,7], 20.17% (45/223) were considered as XDR-TB strains. Most of these strains showed resistance to four drug combination, viz. KM, ETH, OFX & PAS (13/223) followed by KM & OFX (7/223), while 3/223 strains showed resistance to all the drugs i.e. pan resistance (1.34%) used in treatment of MDR cases. Nearly 80% of the XDR-TB strains showed resistance to three or more drugs combination pattern (Table -2).

In the other second line ATT drug resistant pattern, more than four drugs resistance pattern was observed in 30 out of 223 (13.45%) MDR-TB isolates and the maximum resistance was seen in the combination of KM, ETH, OFX and PAS (5.82%) (Table-3).

Table 1: MDR TB strains resistant to second line ATT drugs [n = 223]

Drugs	No. of Resistant *M.tb* strains	Percentage of *M.tb* strains
Ofloxacin	154	69.05
Ethionamide	87	39.01
PAS	61	27.35
Kanamycin	46	20.62
Capreomycin	22	9.86
Amikacin	9	4.03
Cycloserine	7	3.13

PAS = Para-Aminosalicylic Acid
M.tb = *Mycobacterium tuberculosis*
MDR TB = Multidrug resistant tuberculosis

Table 2: Number of XDR-TB strains & its resistance pattern among the MDR-TB strains

Drug patterns	No. of Resistant strains	Percentage of Resistant strains
KM , OFX	7	3.13
KM, OFX, PAS	1	0.44
KM, OFX, ETH	4	1.79
KM, OFX, CM	2	0.89
KM, OFX, ETH, PAS	13	5.82
KM, OFX, AMK, CPM	3	1.34
KM, OFX, ETH, CPM	3	1.34
KM, OFX, AMK, CPM, PAS	1	0.44
KM, OFX, ETH, AMK, CPM	1	0.44
KM, OFX, ETH, CPM, PAS	3	1.34
OFX, CPM	2	0.89
OFX, ETH, CPM	2	0.89
All Resistant	3	1.34
Total	45	

PAS = Para-Aminosalicyclic Acid, KM = Kanamycin,
OFX = Ofloxacin, ETH = Ethionamide, CYS = Cycloserine,
CPM = capreomycin

Table 3: Second line ATT drugs resistance pattern of MDR strains

Drugs	No. of Resistant strains	%age of Resistant strains
KM	1	0.44
KM , ETH	2	0.89
KM, PAS	1	0.44
OFX	58	26.00
OFX, ETH	20	8.96
OFX, PAS	13	5.82
OFX, ETH, PAS	16	7.17
ETH	12	5.38
ETH, PAS	5	2.24
PAS	1	0.44
PAS, CYS	2	0.89
KM, ETH, AMK, CPM	1	0.44
ETH, OFX, CYS, CPM, PAS	1	0.44
ETH, OFX, CYS, PAS	1	0.44
ALL SENSITIVE	44	19.73
Total	178	

PAS = Para-aminosalicylic acid, KM = Kanamycin, OFX = Ofloxacin,
ETH = Ethionamide, CYS = Cycloserine, CPM = Capreomycin,
ATT= Antituberculosis treatment

A total of 44 (19.73%) MDR-TB strains were sensitive to all the tested second line ATT drugs (Table- 4).

Apart from the criteria met for 45/223 XDR-TB strains as already specified by WHO, we found that 79.82% (178/223) strains were resistant to other drug combinations of second line ATT drugs.

Table 4: Status of MDR strains January, 2007-December, 2009 [n = 223]

Type of Resistance	No. of Resistant samples	Percentage of Resistant samples
XDR-TB	45	20.17
Any Resistant	134	60.08
All Sensitive	44	19.73
Total	223	

Status of XDR TB reported in different research publications

Place of study	Year of study	No of MDR TB strains tested	No of XDR resistant strains (%)	Reference No
Italy	1993-2004	83	8(14.3)	9
Germany	1993-2004	43	3(10.3)	9
France	2006	_	4%	22
Iran	2006	113	12(10.9)	23
Hong Kong	2004	75	9(12.01)	24
Industrialsed nations	2000-04	821	53(6.5)	10
Eastern Europe and Russia	2000-04	406	56(13.6)	10
Republic of Korea	2000-04	1298	200(15.4)	10
India	2006	68	5(7.3)	14
India	2008	12	4(33.3)	15
India	2007	326	36(11)	18
India	2007	66	1(1.5)	17
India	2009	211	5(2.4)	16
India	2009	216	7(3.1)	5
Present study	2010	223	45 (20.17)	Present study

DISCUSSION

The present study showed 20.17% of XDR-TB strains amongst a total of 223 MDR- TB strains . The figure being high, it must be emphasized that this was observed in a referral hospital setting.

Data of patients undergoing treatment for MDR-TB in USA showed the presence of XDR-TB as 6.5%, Germany 10.3%, Russia 13.6%, Italy 14.3% and in Hong Kong 12%. Republic of Korea had reported 15.4 % of XDR-TB cases from a total of 1298 MDR-TB strains[9-11]. During 2008, 963 XDR-TB strains were reported to WHO from 33 countries. In January, 2010 also, as many as 58 countries reported XDR-TB strains to WHO.[5]

Previous data from India as observed by unaccredited laboratories showed varying results as 7.3% XDR-TB strains by Mondal *et al* and 33% of XDR-TB strains reported by Singh *et al*, Sharma *et al* reported 2.4% XDR-TB strains and Ramachandra *et al* reported 3.1% XDR-TB strains[4-16,5]**.** The only accredited laboratory TRC Chennai which did population-based survey, reported prevalence of 1.5% XDR-TB[17].

An abstract from Hinduja Hospital, Mumbai presented at American Thoracic Society International Conference held in May 2007 at San Francisco observed that among the total tested 326 MDR-TB strains, 11% were XDR-TB strains[18]. However, these are again hospital-based data and none of these laboratories are accredited, more so for second line drug testing.

Present study showed that MDR-TB is not only resistant to quinolones and aminoglycosides but other second line ATT drugs also.

Kanamycin and capreomycin cross resistance pattern is a common finding ranging from 20% -60% resistance documented in CDC unupdated data and was seen in this study also[19,20].

We observed maximum resistance to ofloxacin 69% [154/223]. This may probably be due to random use of quinolones by many registered and non-registered practitioners for common diseases. This highlights the problem in opting drug regimen to treat MDR cases. Widespread simultaneous usage of many second line ATT drugs for treatment of MDR-TB and fluoroquinolones for treatment of other diseases like upper respiratory tract infection, urinary tract infection, etc., at the same time, in the country may be the reason for the emergence and spread of resistant TB in community. Singh *et al* reported that 7% and 53% of *Mycobacterium tuberculosis* strains were resistant to ofloxacin isolated from category 1 and category II respectively from patients of Kanpur and Agra[21].

Reports from Korea showed 4.4% resistance to fluoroquinolones drug which could be due to lesser usage of the drug in their country[10].

Eighty per cent of the total XDR-TB strains were found to be resistant to three or more second line ATT drugs. Saha *et al* also found that 70% of the total XDR-TB strains were found to be resistant to two or more second line ATT drugs[10]. This may be due to the fact that use of second line ATT drugs is widespread and unchecked.

Despite giving the complete picture of XDR-TB strains, the limitation is that data do not represent the population status. Likelihood of selection bias is there because all the strains were multidrug resistant strains and are not representative sample of the community. This data cannot be generalized for the general population. Some more population-based surveillances can be undertaken. Another limitation of this study is the lack of clinical information of patients because we haven't tracked the record of these patients. Despite these limitations of this study, the existence of XDR-TB in India is well-understood. Its prevention calls for an urgent and rational use of second line Anti-TB drugs and pragmatic management of MDR-TB. Further, more data are required to be generated at the community level.

CONCLUSION

In conclusion, the multidrug resistant TB cases need urgent and timely sensitivity report for second line ATT drugs to help the clinicians start

proper drug combinations to treat MDR-TB patients. More detailed and population-based studies are required to know the burden of XDR-TB strains in community. XDR-TB is throwing an open challenge to clinicians and policy makers as mycobacterium is growing immortal and devastating.

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Original Article

INCREMENTAL YIELD IN SPUTUM SMEAR POSITIVITY BY EXAMINING A SECOND EARLY MORNING SPUTUM SPECIMEN IN FOLLOW-UP PATIENTS ON DOTS: 7 YEAR ANALYSIS OF RNTCP LABORATORY REGISTER

Gita Nataraj[1]*, Swapna Kanade[2]*, Raunak Parikh[3]*, Vijay Khatri[2], Preeti Mehta[4]**, Amita Athavale[4]** and Bamne Arun[5]*****

(Received on 6.10.2010. Accepted after revision on 16.3.2011)

Summary

***Background*:** Patients receiving DOTS undergo periodic follow-up sputum examination, which aids in monitoring response to treatment. Continued or new smear positivity at follow up examination entails extension of intensive phase or change in treatment category and the need for culture and drug susceptibility test.

***Setting*:** Tuberculosis microscopy centre at a tertiary care teaching hospital, Mumbai, India.

***Objective*:** To determine the incremental yield in sputum smear positivity by examining a second early morning sputum specimen in follow-up patients on DOTS.

***Design*:** Retrospective analysis of follow up sputum microscopy results recorded in tuberculosis laboratory register for the period 2002-2008.

***Results*:** During the study period, 5015 follow-up patients submitted two early morning sputum specimens, of which 501(9.99 %) patients were detected to be smear-positive. Out of smear positive patients under study, 324 patients had both specimens positive, 79 patients had only first specimen positive and 98 patients had only second specimen positive. The incremental yield was 1.95 % of total and 19.5 % of smear positives.

***Conclusion*:** Discordant smears were present in nearly a third of patients detected smear positive during follow-up. More than half of these patients were detected only by examining second specimen. The incremental yield by examining the second early morning specimen was 1.95 % of total and 19.5 % of smear positive specimens. It is important to detect each possible smear positive follow-up patient as they are likely to benefit from altered treatment. The inclusion of a second early morning sputum specimen examination is essential to maximize their detection. **[*Indian J Tuberc 2011; 58:* 60-65]**

Key words: **S**mear positivity, Incremental yield, Follow up patients, DOTS

INTRODUCTION

Under the Revised National Tuberculosis Control Programme (RNTCP) for Tuberculosis [TB], sputum smear examination forms the cornerstone of both diagnosis as well as monitoring response to treatment. For monitoring response to treatment, all patients on DOTS are expected to undergo follow up sputum smear examination for acid fast bacilli (AFB) at defined intervals based on their treatment category.[1]

The first follow-up sputum examination is done at the end of intensive phase (IP). It is expected that treatment responders should demonstrate a reduction in bacillary load or absence of bacilli or continued sputum smear negativity. A patient who is smear positive at the end of IP, will receive an additional month of IP drugs following which he / she would be shifted to continuation phase (CP) irrespective of the smear status at the end of extended IP. Patients who are smear positive at or after five months of DOTS are judged to have failed treatment under that treatment category.

The International Union Against Tuberculosis and Lung Diseases (IUALTD) has recommended a single sputum examination for follow-up patients on each occasion and only the smear positives should be

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reconfirmed by examining the second specimen[2]. The Tuberculosis Coalition for Technical Assistance recommends examination of two sputum specimens irrespective of the smear status of the first.[3] Under RNTCP, patients (diagnosis and follow-up) have to submit two sputum specimens which should include at least one early morning specimen.[1] A single positive sputum smear is sufficient to label the patient as "smear positive".[4]

Examination of multiple specimens from the same patient is advocated to improve the sensitivity of detection by microscopy as well as to reduce false positivity, but it is not without limitations. It adds on to the laboratory workload, cost in terms of manpower, equipment and consumables and in high burden laboratories may compromise quality.[5-8] Operational research has therefore been advocated by RNTCP to identify the usefulness of examining additional sputum specimen.[9]

Although studies have shown the advantages of examining only two as against three sputum specimens for diagnosis of pulmonary TB patients, there appears to be a paucity of published data on the examination of one against two sputum specimens in follow up patients.[7, 8, 10, 11] In our centre, during the study period, patients submitted two consecutive early morning (EM) specimens for follow up examination. We wanted to determine the added value by examining a second EM sputum specimen in follow up patients.

AIMS AND OBJECTIVES

The aim of this study was to determine the incremental yield by examining the second EM sputum specimen with the following objectives (i) the number and percentage of follow-up patients detected to be smear positive (ii) the incremental yield from the second EM specimen.

MATERIAL AND METHODS

Seth G.S. Medical College and K.E.M Hospital, a 1800 bedded tertiary care teaching hospital located in F-South ward in Mumbai, India with a microscopy centre, is one of the five such centres in this ward. The F-South municipal ward in Mumbai has a ward population of 4,37,097 with an estimated incidence rate of 1006 TB cases per year.[12]

Permission to carry out the study was obtained from Institutional Ethical Committee and Mumbai District TB Control Society.

A retrospective analysis of data recorded in RNTCP TB laboratory register at the microscopy centre was carried out for the period 2002-2008. For each follow up patient, two EM sputum specimens were processed. Only those patients who had submitted two EM specimens were included in the study.

The protocol of examining two early morning sputum specimens was adopted for the following reasons. In terms of infection control, no safe place could be identified for patients to expectorate, especially with the proximity of the Integrated Counselling and Testing Centre. Being a tertiary care centre, patients are advised other investigations necessitating their visit to the hospital on more than one occasions.

RESULTS

During the study period, a total of 19,199 patients were tested at the AFB microscopy centre,

Table 1: Follow up patients attending microscopy centre

Year	Total follow-up patients tested	Smear positive follow-up patients (%)
2002	622	73 (11.73)
2003	802	61 (7.60)
2004	889	65 (7.31)
2005	798	82 (10.27)
2006	608	84 (13.81)
2007	652	63 (9.66)
2008	644	73 (11.33)
Total	5015	501

of which 5015 (26.12 %) were follow-up patients (Table 1). The follow up number does not include the 30 patients who submitted only one specimen. A total of 501 (9.99 %) smear positive follow up patients were detected. The percentage of smear positive follow up patients detected annually ranged from 7.31% to 13.81%.

Discordant results between first and second smear examination were present in 177 / 5015 (3.53 %) patients with a range of 2.36% - 5.78% per year. The incremental yield obtained by examining the second EM sputum specimen was 98 /5015 (1.95%) with a range 1.12% - 2.89% per year (Table 2).

A further analysis of the discordant smears based on smear grade (Table 3) revealed that discordance was maximum when smears were graded scanty followed by 1+.

Table 2: Year-wise distribution of incremental yield and discordance in follow up patients

Year	Patients tested	Total Smear positive	SSP in both specimens	SSP only in 1st specimen	SSP only in 2nd specimen	Discordant Results (%)	Incremental yield (%)
2002	622	73	37	18	18	36 (5.78)	18 (2.89)
2003	802	61	37	13	11	24 (2.99)	11 (1.37)
2004	889	65	44	08	13	21 (2.36)	13 (1.46)
2005	798	82	62	11	09	20 (2.50)	09 (1.12)
2006	608	84	61	10	13	23 (3.78)	13 (2.13)
2007	652	63	34	12	17	29 (4.44)	17 (2.60)
2008	644	73	49	07	17	24 (3.72)	17 (2.63)
Overall	5015	501	324	79	98	177	98

SSP= Sputum smear positivity

Table 3: Pattern of smear grading in smear positive patients

Result of 1st / 2nd specimen	Total	Specimen 1					Specimen 2				
		Neg	Scanty (%)	1+ (%)	2+ (%)	3+ (%)	Neg	Scanty	1+	2+	3+
Pos /Pos	324	0	54 (16.67)	108 (33.33)	70 (21.60)	92 (28.4)	0	61 (18.82)	98 (30.24)	78 (24.08)	87 (26.86)
Pos /Neg	79	0	40 (50.63)	26 (32.92)	10 (12.66)	3 (3.79)	79	0	0	0	0
Neg /Pos	98	98	0	0	0	0	0	45 (45.91)	41 (41.84)	5 (5.11)	7 (7.14)

Table 4: Disintegrated data of sputum smear positive follow up patients

Year	Total SSP patients	SSP only in 2nd specimen	End of IP		2 months in CP		End of treatment	
			Total SSP	SSP only in 2nd specimen (%)	Total SSP	SSP only in 2nd specimen (%)	Total SSP	SSP only in 2nd specimen (%)
2002	73	18	47	13 (27.66)	21	5 (23.81)	05	0
2003	61	11	38	08 (21.05)	14	2 (14.28)	09	1 (11.11)
2004	65	13	42	08 (19.04)	15	4 (26.66)	08	1 (12.5)
2005	82	09	56	05 (8.92)	17	3 (17.64)	09	1 (11.11)
2006	84	13	49	08 (16.32)	23	3 (17.65)	12	2 (16.66)
2007	63	17	29	11 (41.38)	21	5 (23.80)	13	1 (7.69)
2008	73	17	38	13 (37.93)	20	2 (10)	15	2 (13.33)
Total	501	98	299	66 (22.07)	131	24 (18.32)	71	8 (11.27)

Follow up sputum smear positive patients were further categorized according to the duration of treatment they had received, into end of IP, two months in CP and end of treatment (Table 4). 59.68% (299/501) patients were sputum smear positive at the end of IP, of which 22.07% had only second sputum sample positive. Similarly 18.32% (24/131) of two months in CP and 11.275 (8/71) of end of treatment patients were detected only with the second early morning specimen.

DISCUSSION

The present study is a seven year retrospective analysis of the results of follow up sputum smear examination extracted from the RNTCP TB Laboratory Register with an aim to determine the usefulness of examining the second EM sputum specimen. At each follow up visit, RNTCP recommends examination of two sputum specimens of which at least one should be EM. In the present study, both the specimens examined were early morning. However, since April 2009, as per the new guidelines of RNTCP for submission of specimens, patients visit the microscopy centre just once, having collected both the EM and spot specimens at home.

Smear positivity in follow-up patients was 9.99% (501 / 5015), which is in line with the expected positivity in follow up examination.[13,14] Of these, 98 were detected only by examination of second specimen giving an average incremental yield of 1.9%. Shivakumar *et al* have concluded in their study that repeated follow-up smears are not essential since the incremental yield with a second spot sputum specimen is negligible, provided that the first smear is from early morning specimen.[7] The higher incremental yield in the present study as compared to Shivakumar *et al*, may be attributed to the examination of two EM specimens instead of one EM and one spot. [7] Once patients initiate DOTS, over a period of time and especially at the end of intensive phase, expectoration reduces considerably as does the bacillary load. This is reflected in the quality of the specimen submitted with a tendency to a more liquid consistency as compared to an earlier mucopurulent nature. A poor quality spot specimen is more likely to be negative as compared to an EM specimen from the same patient.

While the incremental yield was 1.95 % (98/5015) of total patients tested, it was 19.5% (98/501) of smear positive patients detected. It is likely that at our testing centre, approximately 10 smear positive

patients per year would have been missed if the second EM specimen was not examined. The detection of an additional 10 smear positive follow up patients annually by examining the second specimen is important for two reasons. One, their IP needs to be extended or treatment category needs to be changed depending on whether the smear positivity is at the end of IP or at or after five months of DOTS. Two, their drug resistant status needs to be ascertained. Even one such case per centre per year needs to be detected since MDR-TB is a national emergency.

If the value of the additional sputum examination is looked in terms of numbers, over seven years it made a difference to 98 patients at an average of 9.9 patients annually at one centre alone, who have likely been benefitted from the extended or changed treatment regimen. The RNTCP TB laboratory register does not have a column to record the duration of treatment taken by follow up patients making it difficult to say how many of the 98 patients required an extension of IP or how many required a category change. Hence details of time since treatment at the time of specimen submission were obtained from our DOTS centre.

Of the 98 patients detected sputum smear positive only with a second early morning sputum specimen, 66 had come at the end of IP, 24 at two months in CP and eight at the end of treatment. Sputum smear positivity only in the second specimen was detected in 22.07% of the smear positive patients coming at the end of IP. These patients therefore could receive an additional month of IP. Examining the second early morning specimen also identified 32 failures (sputum smear positivity at two months in CP and at the end of treatment) whose treatment category would require a change and in whom there is a possibility of Multidrug Resistant Tuberculosis (MDR-TB).

The microscopy centre is a participant in the external quality assurance programme under RNTCP and has performed consistently well during the study period. Majority of the additional smear positive cases detected by examining the second specimen have been graded scanty. Smears with lower grades are more likely to be missed.[15] Given that smear negative patients can also transmit tuberculosis though to a lesser degree, it is important to detect all smear positive patients.[5]

Until such a time that rapid culture and drug susceptibility testing are routinely made available and affordable under the programme, especially for follow-up smear positive patients, the microscopic examination of a second early morning sputum specimen is essential. Insufficient specimen collection should not be a contributory factor for delaying the necessary change in treatment status or early identification of a drug resistant case.

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Original Article

ACUTE SUPPURATIVE PRESENTATION OF OSTEOARTICULAR TUBERCULOSIS IN CHILDREN

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Summary

***Introduction*:** Osteoarticular tuberculosis is known for its atypical presentations. The acute presentation of osteoarticular tuberculosis although mentioned by many observers is seldom seen in practice. We report the varied presentations of acute suppurative presentation of osteoarticular tuberculosis in pediatric age group.

***Methods*:** Our study retrospectively assessed 10 children with osteoarticular tuberculosis who had acute presentation with short history of a few days and signs of acute inflammation.

***Results*:** The age range varied from 3-12 years. The duration of symptoms averaged 14.7 days (range, 4-28 days). Three patients were afebrile at the time of presentation. The diagnosis of tubercular aetiology was made only retrospectively in all the cases. All, except one, were subjected to Fine Needle Aspiration Cytology (FNAC)/ trochar bone biopsy. Diagnosis was based on smear positive for acid fast bacilli (n=3), histopathology (n=5), and on clinicoradiological findings in two cases. The acute exudative pattern was seen in seven and gravity assisted tracking and accumulation of abscess (dependent) in three patients. In eight patients, the FNAC/ trochar biopsy decompressed the lesion initially. Incision and drainage were performed on one case of osteoarticular tuberculosis with clinical presentation of acute pyogenic infection. Another patient of acute exudative pattern was subjected to drainage and debridement in view of impending burst. Both exudative and dependent forms of suppurative presentations of osteoarticular tuberculosis responded well to standard antitubercular chemotherapy. The abscesses resolved within a period of 6-12 weeks.

Conclusion*:** The acute suppurative presentation is a rare and atypical form of osteoarticular tuberculosis. It has close resemblance to acute pyogenic infections or septic arthritis and pose significant diagnostic dilemma for the unwary. A vigilant and methodical approach is the key for managing acute suppurative tubercular presentations. ***[Indian J Tuberc 2011; 58: 66-71]

***Key words*:** Tuberculosis, Pyogenic, Cold abscess, Pediatric

INTRODUCTION

Osteoarticular tuberculosis is known for its atypical presentations.[1] The infrequent occurrence of these forms of tuberculosis poses a diagnostic challenge for the treating clinicians and often results in delayed recognition and treatment. Many a time, the patient has been subjected to unnecessary or even multiple surgeries pending correct diagnosis.[2,3] Acute suppurative presentation is one of the atypical forms of osteoarticular tuberculosis and closely mimics acute pyogenic infection or septic arthritis. We report the varied presentations of acute suppurative presentation of osteoarticular tuberculosis in the pediatric age group.

METHODOLOGY AND RESULTS

We retrospectively analyzed records of 10 children (2005-2009) of osteoarticular tuberculosis who had acute presentation with short history of a few days and signs of acute inflammation (Table 1) (Figure 1). The study was undertaken after prior approval from Institution's Ethical Committee.

The age range varied from 3-12 years. The duration of symptoms averaged 14.7 days (range, 4-28 days). No patient gave history of intake of antitubercular drugs in the past. Three patients were afebrile at presentation. However, they had history of low grade or occasional episodes of fever prior to consultation with us. The initial work-up

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Table 1: Patient data (n=10).

S.No.	Age (in years)	Involved bone/ joint	Abscess Site	Category	Duration of symptoms** in days	Basis of diagnosis
1.	9	Rt. 1st MC	Thumb	acute	21	histopathology
2.	12	L5-S1	Rt. gluteal region	dependent*	28	clinicoradiological
3.	11	Lt. hip	Gluteal region	dependent	28	clinicoradiological
4.	5	Rt. cuboid	Foot lateral aspect	acute	10	histopathology
5.	12	Rt. 5th MT	Foot lateral aspect	acute	7	smear positive for AFB
6.	8	Lt. clavicle	Medial end of clavicle	acute	5	smear positive for AFB
7.	10	Sternum	Lateral end of clavicle	dependent	14	histopathology
8.	3	Rt. humerus lower end	Medial aspect of elbow	acute	4	smear positive for AFB
9.	3	Lt. PP IF, Lt. 5th MT	Index finger	acute	16	histopathology
10.	3	Lt. ankle	Ankle	acute	14	histopathology

** = duration of symptoms at local site.
**Dependent*- tracking and gravity assisted accumulation of abscess
Abbreviations: Rt.-right; MC- metacarpal; Lt.-left; MT-metatarsal; PP-proximal phalanx; IF-index finger, AFB- Acid fast bacteria.

(except case 10) was essentially similar as for acute pyogenic infection or septic arthritis and included complete hemogram, Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), chest x-rays and radiographs of the affected part. Two patients had evidence of old tubercular infection in the past in the form of calcified pulmonary foci. The diagnosis of tubercular aetiology was made only retrospectively in all patients. In one patient with ankle involvement, an arthrotomy was performed in emergency on clinical diagnosis of septic arthritis (Case 10) (Figure 2). The diagnosis of tuberculosis was made on synovial histopathology in this patient. All other patients were subjected to FNAC from non-dependent area. In patients with osseous lesions, a trochar bone biopsy correlating with radiological

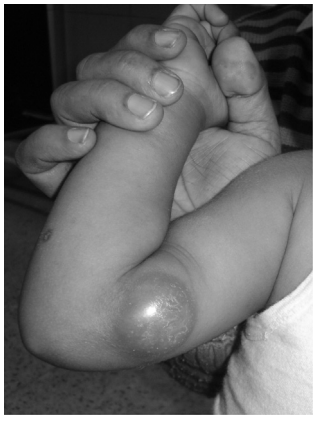

Fig. 1. Case 8. The three year young child presented with acute suppurative tubercular abscess with just four days' history. A smear of the pus aspirated from non dependent area revealed acid fast bacteria.

Fig. 2. Case 10. a) The osteoarticular tuberculosis was not kept in differential diagnosis. The ankle was drained following a clinical diagnosis of acute pyogenic arthritis. The synovial histology revealed tubercular granulation. b) Subsequent plain radiographs taken show irregularity and lytic areas in talus.

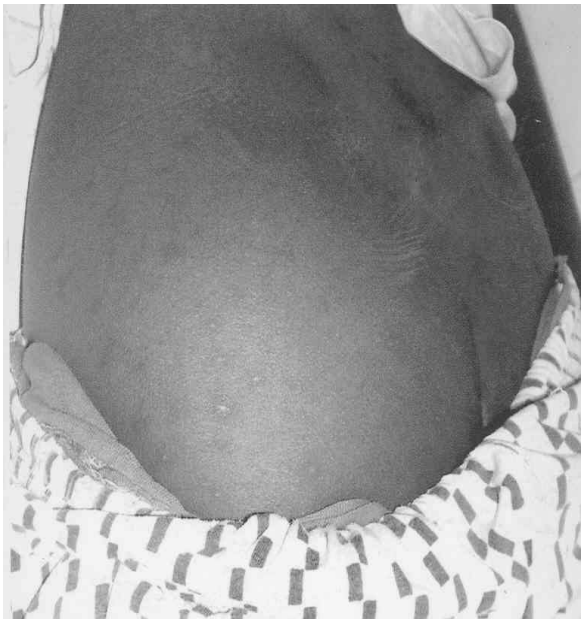

Fig. 3. Case 3. The acute gluteal abscess was a manifestation of left hip tubercular involvement.

lesion was also taken. In cases 2 and 7, the primary was obvious in Magnetic Resonance Imaging (MRI) obtained following suspicion in plain radiographs. In case 3 (Figure 3), the left hip involvement was evident in radiographs of pelvis with both hips. The specimen thus collected was sent for Gram stain, acid fast bacteria stain (Ziehl–Neelsen stain) and histopathology examination. Tubercular diagnosis was based on smear positive for acid fast bacilli (n=3), histopathology including arthrotomy case (n=5), and on clinicoradiological findings in two cases. There were seven patients with acute exudative and three with gravity assisted tracking and accumulation of abscess (dependent*)* (see discussion) pattern (Table 1). In eight patients, the FNAC/ trochar biopsy decompressed the lesion initially. In case 6, in view of impending burst, the abscess was incised and cavity debrided. Two patients with gluteal abscesses were subjected to repeated debulking aspirations (Cases 2 and 3). The cases were treated according to the departmental protocol for management of all osteoarticular tuberculosis cases including spinal tuberculosis with or without neurological deficit. All

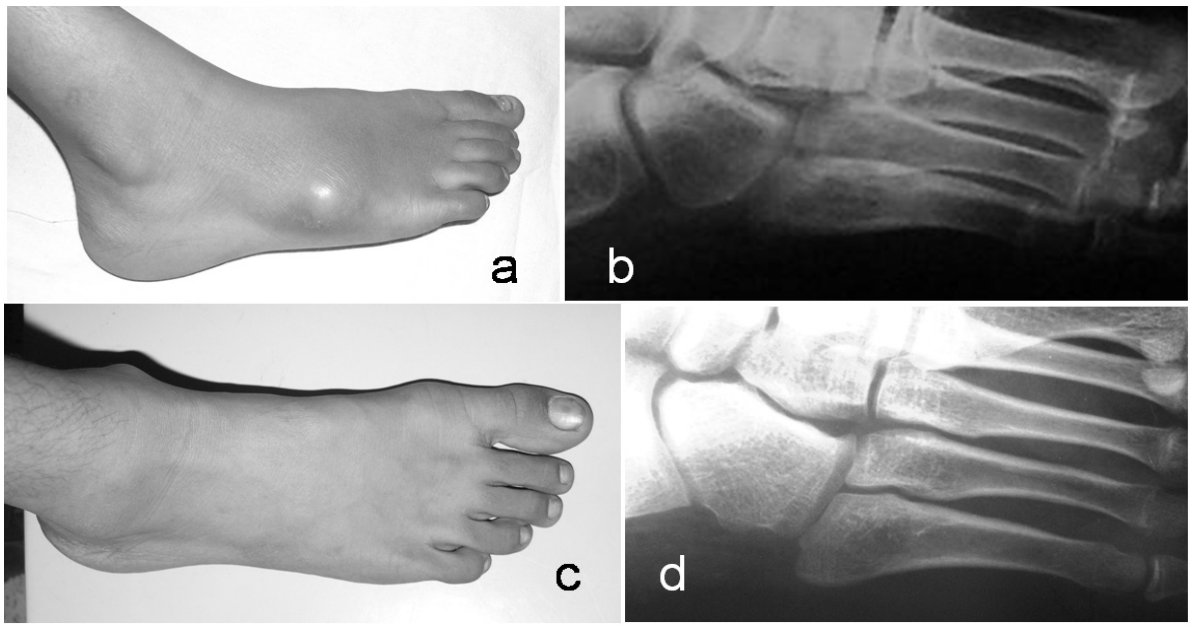

Fig. 4. Case 5. a) Acute suppurative presentation of osteoarticular tuberculosis of 5th metatarsal. b) Plain radiographs showing lytic areas and expansion of base of 5th metatarsal. c) Resolution of abscess following antitubercular treatment in six weeks. d) The lesion in the bone resolved in five months.

patients were placed under Category I and initiated on four drug chemotherapy under DOTS, starting with intensive phase of four drugs for two months (isoniazid, rifampicin, ethambutol, pyrazinamide) followed by continuation phase consisting of two drugs (isoniazid, rifampicin) for six months. The treatment was extended to a total of 12 months by continuation of two drugs (isoniazid, rifampicin) further for six months.[4-8] The acute exudative patients were given splintage at involved site. In dependent pattern, the management was based on disease activity of primary site. In all cases, the systemic signs (e.g. fever) responded by average of three weeks and the abscesses resolved by 6-12 weeks on standard antitubercular chemotherapy (Figure 4).

DISCUSSION

Classically, tuberculosis has been associated with a chronic course and "cold" abscesses composed of products of liquefaction, serum, leucocytes, granulation tissue, necrotic material, bone debris and tubercle bacilli.[4] Tubercular abscess is described as warm when compared with an acute pyogenic abscess. In certain atypical conditions, the tubercular presentation closely mimics an acute pyogenic infection or septic joint. Acute suppurative presentations of tuberculosis have been described with involvement of lymph nodes, thyroid, breast etc., but infrequent with osteoarticular tuberculosis.[9-11] The suppurative presentations of tuberculosis may have very similar duration of symptomatology (days-weeks) and the systemic signs corresponding to an acute pyogenic infection.[1,3] The abscess aspirate characteristics are typically misleading and may not reveal tubercle bacilli.[1] The diagnostic dilemma is further intensified in small children where incidence of septic arthritis and acute pyogenic infections is high in developing countries. Many a time, the acute abscess has been incised and drained in mistaken diagnosis of acute pyogenic infection (Case 10) (Figure 2).[2]

The atypical acute suppurative presentation may be encountered in three situations of osteoarticular tuberculosis. Firstly, there may be acute exudative response associated with tuberculosis. This pattern is believed to be hypersensitivity response to an already existing old tubercular lesion in body.[12] The old lesion may be untreated or partially treated but contained by natural immunity.[13] Many of these patients may exhibit scar marks of old healed tuberculosis. This variety is manifested more with involvement of superficial bones and joints such as phalanges, metacarpals, clavicle, sternum, shoulder joint, etc. We had seven patients demonstrating this variety (Table 1) (Figures 1, 4). We found no specific predilection for any joint or bone in acute suppurative presentation of tuberculosis. Teklali *et al* has described this type of 'acute' presentation in nine patients in his series of 106 children with osteoarticular tuberculosis collected over a period of 21 years but provided no further details.[13]

Second variety is a complication of gravity assisted tracking and collection of cold abscess, burst impending. As a large amount of cold pus accumulates in preformed lax tissues, it causes pressure necrosis of the overlying subcutaneous tissue and skin. The soft tissue produces a local inflammatory response clinically appreciable as "hot" abscess. In this pattern, usually the formation of pus takes place at a different site from abscess accumulation such as spine, hip etc. The pus migrates from primary site to a distant site such as buttock, knee, neck, etc. (Figure 3). The systemic signs vary according to the disease activity at the primary site and high grade fever may not be present. Associated spasm at local site may also be absent. In the current series, dependent variety was seen in three patients.

A third extremely rare scenario is concomitant suppurative and tubercular infection.[3,13,14] Opara *et al* described a knee joint involvement in a 23-year-male where the superficial popliteal abscess was due to popliteal lymph node suppuration caused by staphylococcus aureus and knee sepsis was due to tuberculosis.[3] Clinical distinction between acute pyogenic and tubercular infection is extremely difficult in this variety and frequently missed.[3] We did not have any such case in our series.

The three different patterns of acute suppurative presentation associated with osteoarticular tuberculosis demand awareness of this atypical form, precise and methodical clinical assessment and support of dedicated laboratory services to aid in diagnosis. We suggest collection of samples for Ziehl–Neelsen staining prior to drainage procedure when suspicion for tuberculosis is high or areas where tuberculosis is in endemic proportions. The smear for acid fast bacteria reveals the diagnosis early in many cases. In patients with obvious radiological lesion in bone, FNAC/ trochar biopsy serves the dual purpose of abscess decompression and tissue for diagnosis. Synovial biopsy should always be taken if arthroscopic or open drainage of acute arthritis is performed when risk factors are present. The various risk factors in children described are recent tuberculosis contact, previous pulmonary tuberculosis, malnutrition, poor sanitation, overcrowding, exanthematous fevers, diabetes, trauma, previous steroid therapy, and immunodeficiency.[3,8] In dependent pattern, investigation of primary site is advocated. Once diagnosed, the acute suppurative presentations of osteoarticular tuberculosis respond well to standard antitubercular chemotherapy.[3,12-14] In case of large collection, antigravity aspirations of the abscess are helpful.[7] Some clinicians have also advocated debridement of tubercular abscess.[4,6-8] We recommend surgical drainage of abscess and debridement of the cavity in cases of an impending burst not responding to aspirations or when pressure effects of abscess are problematic.

The limitations of this retrospective study are its small sample size and retrospective diagnosis of the pathology. Quantitative assessment of the various microbiological parameters such as organism's virulence, host immunity and mediators of inflammation

cascade, etc., would provide insight into pathogenesis of this atypical presentation of the disease.

CONCLUSION

The atypical acute suppurative presentations of osteoarticular tuberculosis do exist. Unless specifically considered, this diagnosis will be missed with the typical aerobic and anaerobic cultures. For all cases of suppurative abscess, a high index of suspicion for tuberculosis should be maintained, especially when there are risk factors at presentation or there is failure to achieve an adequate response to appropriate antibacterial treatment.

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Original Article

COMPARISON OF ZIEHL NEELSEN & AURAMINE O STAINING METHODS ON DIRECT AND CONCENTRATED SMEARS IN CLINICAL SPECIMENS

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Summary

Background: In developing countries like ours with a large number of tuberculosis (TB) cases and limited resources, the diagnosis of TB relies primarily on smear microscopy for Acid Fast Bacilli (AFB) but its sensitivity is limited in paucibacillary cases.

Aim: To evaluate the increase in efficacy of smear microscopy when smears are prepared from clinical samples after concentration by Petroff's method and stained by Auramine O (AO) fluorescent dye as against Ziehl Neelsen (ZN) staining of similar taking culture as the gold standard.

Methods: Smears were prepared from 393 clinical samples both by direct and after Petroff's concentration and examined by fluorescent microscopy and Ziehl Neelsen method .The concentrated material was also cultured on Lowenstein Jensen media and the results of the two microscopy methods were compared with the culture results taken as the gold standard.

Results: Mycobacterial growth was detected in 137(35.77%) specimens, out of which three were non-tubercular mycobacteria. Using culture as the reference method, the sensitivity of direct staining was 55.55% for ZN and 71.85% for AO. Direct fluorescent microscopy detected 9.29% paucibacillary sputum samples that were missed on ZN staining. On concentration, the sensitivity increased by 6.67% for ZN and 11.11% for AO. The sensitivity of AFB smear microscopy increased by 27.41% and was statistically significant (p=<.001) when both methods were combined. The specificity was 99.19% for both ZN and AO.

Conclusion: Fluorescent microscopy has higher sensitivity and comparable specificity which is further enhanced by concentration. Now with the advent of newer inexpensive Light Emitting Diode (LED) based fluorescent microscopes (FM), which are easier to use, fluorescent microscopy can be widely used even in peripheral laboratories where culture facilities are not available. **[*Indian J Tuberc 2011; 58:* 72-76]**

Key words: Ziehl-Neelsen Staining, *Mycobacterium tuberculosis*, Auramine O.

INTRODUCTION

The global burden of disability and death due to tuberculosis (TB) is immense. The expanding HIV epidemic has further increased the morbidity and mortality due to HIV-TB co-infection. India accounts for one- fifth of the world's new TB cases and two-third of the cases in South East Asia [1, 2]. An estimated 1.9 million cases occur annually and around 0.9 million have sputum positive pulmonary TB [3]. In recent years, several automated culture systems and molecular techniques have been developed for the diagnosis of TB which have reduced the turnover time for detection of AFB but are costly and are not suitable for routine use in low-middle income countries[4]. AFB smear microscopy using conventional light microscope still remains the mainstay for diagnosis and monitoring treatment of TB as it is simple, inexpensive, widely applicable and highly specific for TB in endemic countries [5-7], but as the sensitivity of direct AFB smear is low, detecting only if 10^5 bacilli are present per ml, there is an urgent need to improve the sensitivity of AFB smear microscopy. Various techniques like concentration by sodium hypochlorite or sodium hydroxide, sedimentation of sputum using chemicals, fluorescent microscopy, etc., have been tried which have increased the sensitivity by 10-23%[8]. However, these techniques have been tried only one at a time. Therefore, the

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Original Article

DOES CSF COMPOSITION PREDICT SHUNT MALFUNCTION IN TUBERCULOUS MENINGITIS?

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Summary

Background: Hydrocephalus secondary to tuberculous meningitis (TBM) continues to be a challenging condition to treat for neurosurgeons in developing countries. Shunt complications are reportedly more frequent in patients undergoing ventriculo-peritoneal shunt in patients with TBM than in those undergoing shunt surgeries for other causes.

Aim: The aim of this study was to evaluate the relationship of cerebrospinal fluid (CSF) composition on shunt malfunction.

Methods: We compared the CSF composition of 53 patients who had shunt malfunction during a five year period with that of 137 matched controls.

Results: Patients who had shunt malfunction had a significantly higher concentration of CSF protein. The CSF cellularity and glucose concentration did not have any significant bearing in predicting shunt malfunction. Patients with CSF protein concentration of more than 200 mg/dL had a four times higher risk of having shunt malfunction than those with a concentration of less than 100 mg/dL. Patients with CSF protein in the 100-200 mg/dL range represent an intermediate zone.

Conclusion: To conclude, patients with CSF protein concentration of more than 200 mg/dL have a significantly higher risk of shunt malfunction and hence have to be followed up closely. **[*Indian J Tuberc 2011; 58: 77-81*]**

Key words: Tuberculous meningitis, Hydrocephalus, Ventriculo-peritoneal shunt, Shunt malfunction.

INTRODUCTION

Tuberculosis remains a major public health problem in the developing countries and with the advent of AIDS, use of immunosuppressant medications and population migration, it is increasingly being seen in the developed countries as well[1]. Though about 5% of tuberculous infections involve the CNS[2], it is important to diagnose and treat them because of the potentially devastating complications and high rate of morbidity and mortality due to tuberculous meningitis (TBM)[3].

Hydrocephalus is the most common complication of tuberculous meningitis seen in up to 87% of patients with tuberculous meningitis; children having a higher prevalence than adults[4]. There have been various studies analyzing the prognostic factors predicting clinical outcome in tubercular meningitis with hydrocephalus[5]. Cerebrospinal fluid (CSF) diversion in the form of ventriculo-peritoneal (VP) shunt is the mainstay in the management of hydrocephalus[3]. Due to various factors, especially the protein content, shunt malfunction is a major problem in these patients. The rates have been significantly higher than those done for other pathologies. Shunt revision carries additional morbidity and cost. Agarwal *et al*[4] reported a shunt malfunction rate of 16% and a shunt infection rate of 14% in their study. There have been no studies predicting the incidence and factors associated with shunt malfunction in patients with hydrocephalus due to tuberculous meningitis. In this study, we analyzed the relationship of CSF composition in predicting shunt malfunction in these patients.

MATERIAL AND METHODS

This retrospective study was conducted at the National Institute of Mental Health And Neurosciences (NIMHANS), Bangalore, India. It included all patients with TBM with hydrocephalus who presented with ventriculo-peritoneal shunt malfunction and underwent shunt revision during 2004-2008. Shunt malfunction was defined as non-functioning of the shunt for causes other than infection and malposition. Patients with

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shunt infection and shunt malposition were excluded from the study as these are established factors of shunt malfunction. Shunt infection was diagnosed when a patient presented with an infected wound or CSF analysis showing polymorphic pleocytosis with low glucose or when the shunt tube was exposed.

Age and sex matched controls were taken from patients who had undergone successful VP shunt surgery for TBM during the same period. The CSF composition was studied in both the study groups.

The diagnosis of TBM was made by cytochemical analysis of the CSF obtained by lumbar puncture in all the patients. All patients were started on standard Anti-Tuberculous therapy (isoniazid, rifampicin, pyrazinamide and ethambutol) with steroids (dexamethasone) after the diagnosis. Shunt malfunction was diagnosed by clinical examination, CT scan of the head and shunt tap whenever deemed necessary. Only the patients with shunt malfunction due to shunt tube block were included in the study, whereas those with improper shunt position and shunt infection were excluded from the study. The CSF profiles of patients who had shunt malfunction and those of controls were studied from the available records from case files. The various variables studied were CSF cell counts, glucose and protein content.

Statistical analysis

Statistical analysis was performed with commercially available software (SPSS 16.0; SPSS, Inc.). Analysis was done using the independent t-test and multivariate logistic regression using the various CSF parameters.

RESULTS

A total of 449 VP shunt surgeries were performed in 432 patients for hydrocephalus due to tuberculous meningitis. Of these, 70 shunt malfunctions were observed in 53 patients. There were 32 males and 21 females. The age of the patients ranged from one year to 40 years with a mean of 15 years. Follow-up ranged from one month to six years with a mean of 24.6 months. A shunt malfunction rate of 15.6% was observed in our series. Of the patients who had undergone successful shunt surgery for TBM, 137 patients were selected as controls after matching the age, sex and clinical grade.

The mean CSF cell count in the group with shunt malfunction was 125.17 and that in the group without shunt malfunction was 116.66 cells. The mean values of CSF glucose concentration in both the groups were 49.68 mg/dL and 54.72 mg/dL respectively. The mean CSF protein concentration observed in both the groups was 311.85 mg/dL and 134.84 mg/dL respectively (Table 1).

There was no significant correlation between the CSF cell counts and risk of shunt malfunction. The same result was obtained when CSF glucose was analyzed (Table 1).

Table 1: CSF parameters studied in case and control groups

CSF parameter	Shunt Malfunction group (n=70) Mean (range)	Control Group (n=137) Mean (range)	p
Cell Count (cells)	125.17 (12-2090)	116.66 (10-8000)	0.93
Glucose (mg/dL)	49.68 (5-133)	54.72 (4-254)	0.30
Protein (mg/dL)	311.85 (44-4430)	134.84 (8-1089)	0.005

Table 2: Distribution of patients into three groups according to protein values

Protein values	n	Shunt patent (n)	Shunt malfunction (n)	Odds ratio	Confidence interval	p
< 100 mg%	109	89	20			
100-200 mg%	39	26	13	2.23	0.98-5.07	0.57
> 200 mg%	59	22	37	4.05	1.86-8.09	< 0.001

The total number of patients was divided into three groups: (1) patients whose CSF protein concentration was less than or equal to 100 mg/dL, (2) patients whose CSF protein concentration was between 100 and 200mg/dL, and (3) those with protein concentration above 200mg/dL. Analysis was performed using the Pearson's Chi square test followed by logistic regression analysis. It was observed that the group of patients whose CSF protein concentration was more than 200 mg/dL had a four times increased risk of having shunt malfunction than the group with CSF protein concentration of 100 mg/dL or less (P<0.001). The patients with CSF protein concentration between 100 and 200 mg/dL were in the intermediate zone. They had a risk of shunt malfunction two times greater than those with a concentration of less than 100mg/dL; however the difference was not significant (P=0.57) (Table 2).

On analyzing CSF protein concentration with the independent T test, there was a significant correlation between high protein concentration and the risk of shunt malfunction. It was observed that higher the CSF protein concentration, greater is the risk of shunt malfunction.

DISCUSSION

Complications of shunt surgery are reportedly higher in patients with tuberculous meningitis than in patients with other conditions. Malnutrition, higher protein content and cellularity in the CSF have been proposed to be predisposing factors to shunt malfunction[6]. In a study by Palur *et al*[5], 26 of the 114 (22.8%) patients underwent shunt revision. In a series of 37 children, Chatterjee *et al*[7] reported a shunt infection rate of 15.6% and a shunt revision rate of 43.8%. In the same study, 18.75% patients required multiple shunt revisions. Lamprecht *et al*[8] reported a shunt complication rate of 32.3%, with shunt infection and shunt obstruction each occurring in 13.5% of cases.

A number of studies have analyzed prognostic factors for long term outcome following shunt surgery. Palur *et al*[5], in a series of 114 patients reported that age, duration of altered sensorium, CSF cell count, CSF protein concentration, shunt revisions have no significant effect on the long term outcome. Clinical grade at admission was the only predictor of long term outcome. Misra *et al*[9] identified age, clinical grade, presence of cranial nerve palsies and severity of hydrocephalus as prognostic variables in their study. Srikantha *et al*[10] in a study of 95 patients concluded that age and duration of altered sensorium predict short-term outcome, while GCS score at presentation predicts long-term outcome after VP shunt placement.

A variety of abnormalities in CSF composition has been described in patients with tuberculous meningitis[11]. Classically, the fluid is clear or slightly opalescent. In patients with severe accompanying vasculitis, hemorrhagic fluid may be observed. When tuberculous CSF is allowed to stand for a short time at room temperature or in the refrigerator, a cobweb-like layer may form on the top of the specimen. This web is the classic "pellicle" of

tuberculous meningitis, which results from the high fibrinogen concentration in the fluid along with the presence of inflammatory cells.

Other CSF abnormalities include elevated opening pressure, lymphocytic pleocytosis (10 to 500), elevated protein concentration in the range of 100 to 500 mg/dL, and decreased glucose concentration. A moderate pleocytosis in the CSF is characteristic of tuberculous meningitis. Counts are generally in the range of 300-500cells/cubic mm, although very high counts are seen occasionally[12]. In the initial stages of tuberculous meningitis, both lymphocytic and polymorphonuclear predominance is seen, although sequential CSF specimens usually show conversion to a lymphocytic picture over several weeks. CSF protein is elevated in most patients with tuberculous meningitis with most series citing a median value of 150-200mg/dL. It is observed that the protein values tend to rise with sequential samples from untreated patients. Very high values are more commonly seen in patients with spinal block[12].

We hypothesize that a high CSF protein concentration resulting in formation of a similar cobweb-like layer as seen *in vitro* within the shunt tube may cause resistance to CSF flow and may result in shunt block. Intermittent drainage of CSF with stasis of the CSF within the shunt tube may be an additional factor promoting the formation of a barrier to CSF flow. One additional factor may be that increased CSF protein levels reflect an increased level of meningeal inflammation and this would result in greater post tubercular inflammatory sequalae such as formation of adhesions and scars which may block the shunt openings.

It was found in our study that high CSF protein concentration is associated with increased risk of shunt malfunction and the association was significant ($P<0.05$). Further, patients with CSF protein concentration of more than 200 mg/dL stood a four times increased risk of having a shunt malfunction than those with a protein concentration of 100 mg/dL or less in the CSF. Those in the CSF protein range of 100-200mg/dL were in the intermediate zone. Possibly, presence of other confounding factors, not as yet determined, influences shunt function.

There are a few limitations in our study. Our study is a retrospective analysis of data in case records. Secondly, our study had not taken into account clinical parameters such as nutrition of the patient. Certain radiological characteristics such as the degree of hydrocephalus and presence of basal exudates, which may play a role in the development of shunt malfunction as well, need to be elucidated by further analysis.

CONCLUSION

A high CSF concentration of proteins (>200 mg/dL) predisposes patients with tuberculous meningitis undergoing VP shunt for hydrocephalus to develop shunt block leading to shunt malfunction. CSF glucose concentration and cellularity do not have any role in the development of shunt malfunction. This subgroup of patients (> 200mg/dL) requires a more aggressive follow-up to diagnose malfunction and thus prevent morbidity/mortality associated with it.

ACKNOWLEDGEMENTS

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A CASE OF ADRENAL TUBERCULOSIS WITH PULMONARY TUBERCULOSIS

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A 56-year-male presented in outpatient department with history of constipation for the last 15 days, one episode of vomiting and urinary retention since afternoon. He is a known case of diabetes mellitus and hypertension for the last two years on oral hypoglycaemic drugs and antihypertensive medications.

Investigations showed Hb 10.4 gm%, TLC 15,800/mm3, P 90%, L07%, E 02%, M 01%, serum urea 85mg%, serum creatinine 2.1 gm%, serum sodium 117 meq/L, serum potassium 3.9 meq/L, RBS 457 mg%, BP 140/90 mmHg in right arm supine position, PR 78/min, regular. USG abdomen showed a well-defined large solid lesion of heterogeneous echo-texture with a few necrotic areas within it with size 11.5X10 cm in retro-peritoneum above kidney suggestive of adrenal mass. He was treated symptomatically with human regular insulin on sliding scale, urinary catheterization, electrolytes replacement, and prophylactic broad spectrum antibiotics. Contrast enhanced computed tomography of abdomen with lung sections (Figure 1) showed evidence of a large heterogeneous left adrenal mass of size 10X 8.2 cm displacing kidney inferiorly, fat plane between upper pole of kidney and mass was maintained with extensive airspace opacification with air bronchogram noted in right middle lobe and basal segment of lower lobe of right lung and subcentrimetric mediastinal lymphadenopathy. CT guided fine needle aspiration cytology from adrenal mass was done suggestive of tubercular inflammation. Patient was started on four drug regimen including rifampicin, isoniazid, ethambutol and pyrazinamide as per Revised National Tuberculosis Control Programme and follow up computed tomography scan of abdomen and lung after two months (Figure 2) showed significant resolution of lung infiltrations and left adrenal mass with size 7.2 X 5 cm. Patient was advised to continue anti-tubercular treatment for a total period of six months with regular follow up.

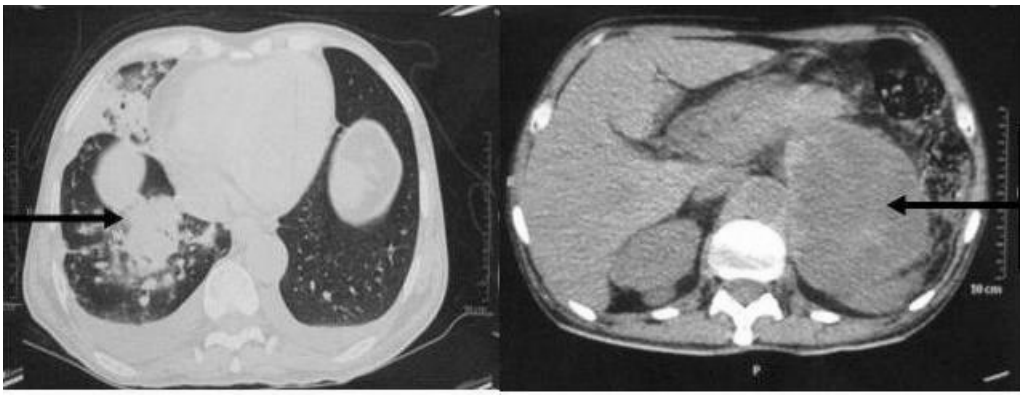

Figure 1: CT Thorax with Abdomen showing large adrenal mass with infiltrations in right lung.

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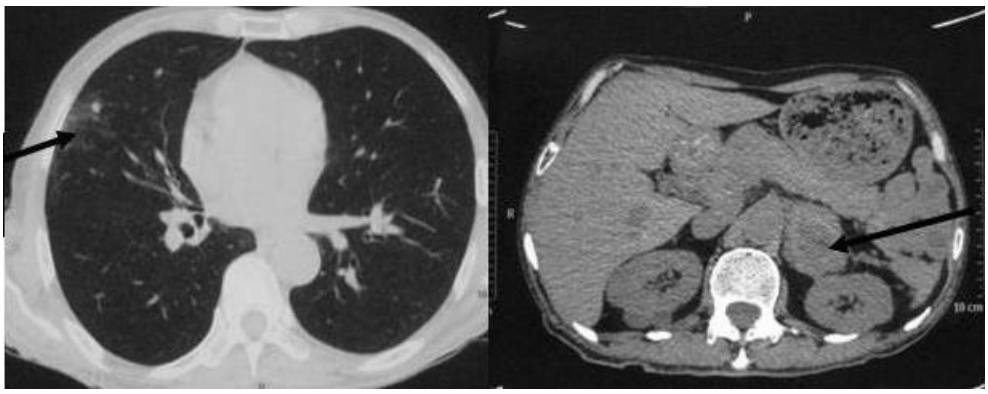

Figure 2: Follow up CT Thorax with Abdomen showing resolution of adrenal mass and infiltrations in right lung.

Adrenal tuberculosis is the most common cause of adrenal insufficiency (Addison's disease)[1]. Adrenal tuberculosis manifests as unilateral or bilateral adrenal enlargement, central necrosis, and calcifications[2]. Guo *et al* compared the contrast enhanced CT scan features of the adrenal glands in 42 patients with adrenal tuberculosis. Of these patients, 91% (38) had bilateral enlarged adrenals and 51% (21) had enlarged glands with preservation of contours[3]. Clinical differential diagnosis included histoplasmosis, blastomycosis, adrenal metastases, primary adrenal tumours and opportunistic infections related to HIV/AIDS[4]. When the disease is treated adequately, adrenal atrophy with calcification may be seen[5].

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Case Report

PULMONARY EMBOLISM IN CASES OF PULMONARY TUBERCULOSIS: A UNIQUE ENTITY

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Summary: Pulmonary tuberculosis is very prevalent in developing countries but its thrombogenic potential is a new entity. There are reports stating the relation of Deep Vein Thrombosis (DVT) with severe forms of tuberculosis but no literature is available for correlation of pulmonary tuberculosis and pulmonary embolism. We are presenting series of five patients with different forms of tuberculosis presenting with pulmonary embolism having no risk factor for hypercoagulability. Also, serum protein C, protein S, antithrombin and factor V levels were normal in all. We are highlighting an unreported phenomenon so that high suspicion, adequate prophylaxis and prompt management of pulmonary embolism can play a vital role in the survival of this subset of patients. **[*Indian J Tuberc 2011; 58:* 84-87]**

Key words: Tuberculosis, Pulmonary embolism

INTRODUCTION

Tuberculosis continues to remain challenging with a variety of complications. Haematological complications have been observed with pulmonary, extrapulmonary and disseminated tuberculosis which usually reverse with antitubercular drugs[1]. DVT is clinically observed and can be confirmed with laboratory methods in 3-4% of patients with pulmonary tuberculosis[2]. Though DVT and pulmonary embolism encompass one disease entity, it occurs about three times more often than pulmonary embolism.

CLINICAL RECORD

1 A 22-year-male presented with fever of one month, swelling and pain in left thigh since 15 days and sudden onset of breathlessness of five hours. He was in shock, tachypnoeic, had raised jugular venous pressure with S1Q3T3 pattern on ECG suggestive of acute cor-pulmonale. Routine investigations were normal with Erythrocyte Sedimentation Rate (ESR) - 110 mm 1st hr Westergren. D-dimer was raised (12mg/l) and venous doppler revealed large thrombus in left iliofemoral vein. Echocardiography showed dilated right atrium, right ventricle, moderate tricuspid regurgitation and pulmonary artery hypertension; Pulmonary Artery Systolic Pressure (PASP): 50mmHg suggestive of pulmonary embolism. Additionally an intracardiac vermicular echogenic mass attached to the interatrial septum was seen. Trans Esophageal Echocardiography (TEE) revealed a large (6x7 mm) intracardiac thrombus straddling across patent foramen ovale (Fig. 1). Pulmonary Angiography showed thrombus in right pulmonary artery (Fig. 2). Systemic thrombolysis with streptokinase was done followed by heparin infusion overlapped with oral anticoagulation. He stabilized but continued to remain febrile and developed left-sided exudative pleural effusion. Polymerase Chain Reaction of pleural fluid revealed mycobacterium species. He was discharged on anti-tubercular therapy and oral anticoagulants. Follow up TEE after two months showed complete resolution of intracardiac thrombus.

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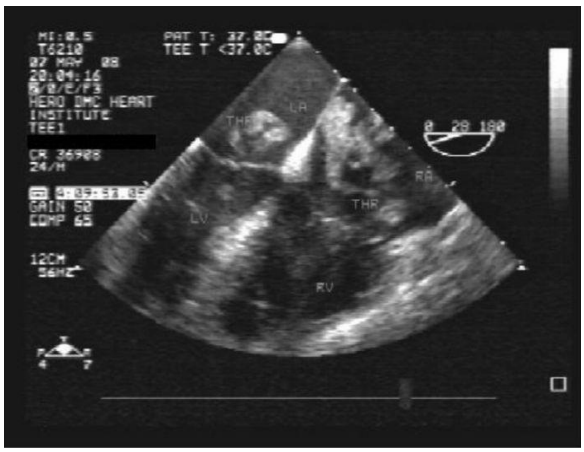

Fig. 1: Trans Esophageal Echocardiography (TEE) showing a large (6x7 mm) intracardiac thrombus straddling across patent foramen ovale.

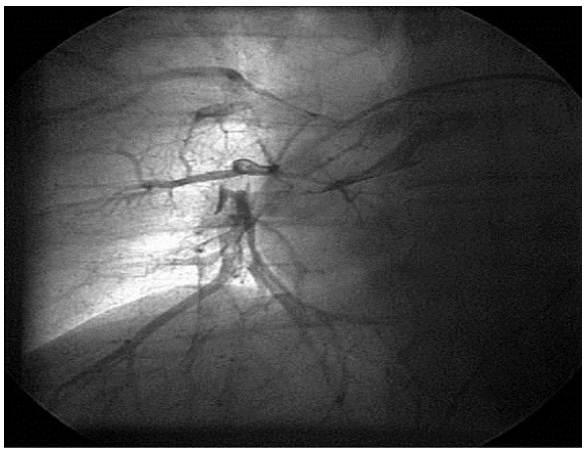

Fig. 2: Pulmonary angiography showing thrombus in right pulmonary artery.

Table showing salient features of the cases

Case	Age (yrs)	Sex	Colour Doppler (Venous)	Echocardiography	Pulmonary Angiography	Type of Tuberculosis
1	22	Male	Large thrombus in left ileofemoral vein	Dilated right atrium & ventricle, Moderate tricuspid regurgitation, Pulmonary artery hypertension, (PASP = 50 mm Hg) Intracardiac vermicular echogenic Mass attached to interatrial septum	Thrombus in right pulmonary artery	Left-sided pleural effusion
2	35	Male	_	Dilated right atrium & ventricle, Pulmonary artery hypertension, (PASP = 52 mm Hg)	Bilateral pulmonary embolism	Cervical lymphadenopathy
3	40	Male	_	Dilated right atrium & ventricle, Pulmonary artery hypertension, (PASP = 62 mm Hg)	Thrombus in right main pulmonary artery with wedge infarct	Miliary tuberculosis
4	47	Male	Large femoral venous thrombus in left leg	_	_	Pulmonary tuberculosis (sputum positive)
5	36	Male	_	_	Thrombus in segmental vessels of both pulmonary arteries, right-sided pulmonary infarct, right-sided pleural effusion, superior vena cava thrombus	Pulmoary tuberculosis with right-sided pleural effusion

2 A 35-year-male, presented with fever, chest pain and breathlessness of two months. He had significant cervical lymphadenopathy. Routine investigations and coagulation profile were normal. Echocardiography was showing pulmonary artery hypertension with PASP of 52 mmHg and RA/RV dilatation. Computed Tomography Pulmonary Angiography (CTPA) was showing bilateral pulmonary embolism. He was thrombolized and still had low grade fever. Fine Needle Aspiration Cytology (FNAC) followed by biopsy of lymph node showed presence of granulomas with caseous necrosis suggestive of tuberculosis.

3 Another patient, 40-year-male presented with cough, expectoration and right-sided chest pain for one month and breathlessness for two weeks. He was hemodynamically stable. Chest radiograph was showing ill-defined haziness with reticular markings in right upper zone and miliary mottling. Mantoux test was positive. Echocardiography revealed dilated RA/RV and PASP of 62 mmHg. CTPA showed pulmonary thrombus in right main pulmonary artery with wedge infarct. He was thrombolized and started on antitubercular therapy. He was stable in follow up after two months.

4 Another 47 year-male, known case of diabetes mellitus type II and sputum positive pulmonary tuberculosis on treatment for last two weeks, presented with acute onset dyspnea and swelling of left leg. He was in shock with BP – 80/60 mmHg, pulse – 180/min and raised JVP. On auscultation, course crepitations over left infrascapular area were present. Chest radiograph had homogenous opacity at left mid zone and normal cardiac size. Routine investigations and coagulation profile were within normal limits. ECG was showing S1 Q3 T3 pattern with right axis deviation. Venous doppler lower limbs revealed large femoral venous thrombus in left leg. He was taken up for mechanical breakdown of pulmonary thrombus and started on thrombolytic therapy followed by anticoagulants and discharged in stable condition after two weeks.

5 A 36-year-male, known case of pulmonary tuberculosis on treatment for the last three months presented with chest pain and acute onset dyspnea. On examination, BP – 90/70 mmHg, pulse – 138/min and JVP was raised. On auscultation, bilateral crepts were present with S3 gallop. Routine investigations were normal. Troponin T was positive by kit method. ECG was showing sinus tachycardia. Chest radiograph had evidence of pleural effusion on right side. CTPA showed segmental vessel of both pulmonary arteries filled with thrombus, right-sided pulmonary infarct, right pleural effusion and SVC thrombus and patient was thrombolyzed with urokinase for 24 hours followed by anticoagulation therapy. Pleural fluid analysis showed exudative effusion with lymphocytic predominance and Adenosine Deaminase (ADA) of 90 U/L. He was discharged on antitubercular drugs in stable condition.

DISCUSSION

Pulmonary tuberculosis is one of the most prevalent diseases mainly infecting people of developing countries. If advanced, its complications are vast to counteract. However, haemostatic complications are very rare and thrombogenic potential of tuberculosis is not frequently documented in literature, especially with reference to pulmonary embolism.

In 1950, pulmonary embolism was found 27 times in 111 subjects of active tuberculosis (24.3%) from 634 autopsies compared to (23.1%) incidence of pulmonary embolism in the entire series[3]. After that, no mention of this important association has figured in literature for the last six decades.

Deficiency of Antithrombin III, protein C and protein S and elevated plasma fibrinogen levels, increased platelet aggregation seems to induce hypercoagulable state in tuberculosis and improves with treatment[4]. It has also been described that activation of endothelial cells occurs in response to various pathophysiological stimuli resulting in expression of endothelial proteins that change the normally non-thrombogenic internal surface of the

vessel to a thrombogenic surface favouring thrombosis[5]. Severe pulmonary tuberculosis is sometimes complicated by DVT and there are few case reports of thrombosis occurring in unusual sites like cerebral venous sinuses[6,7]. The problem is more in critically ill patients with tuberculosis because the rate and degree of stimulated platelet aggregation are increased in severe disease which creates an additional pre-requisite for progression of microthrombogensis.

Vaideeswar P *et al*[8] demonstrated aortic thrombosis in 16.6% patients of tuberculosis in a series of 30 patients. Lang and colleagues[9] have demonstrated increased levels of type I plasminogen activator inhibitor and tissue factor in a 53-year-old male who underwent right upper lobectomy for tuberculosis. Some studies have even demonstrated a possible association between DVT and use of rifampicin with a relative risk of 4.74 in patients of tuberculosis on treatment with rifampicin[10].

CONCLUSION

Our case series highlights the occurrence of pulmonary embolism with pulmonary and disseminated tuberculosis, an entity which was rarely taken into serious consideration. We emphasize the potential seriousness of this unreported phenomenon so that it may play a definite role in long term survival in patients of treatable diseases like tuberculosis.

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DISCUSSION

Invasive fungal infections are increasingly important as causes of morbidity and mortality, especially among immunocompromised patients.The clinical importance of Candida species, Aspergillous sp and Zygomyces is well recognized.Disseminated infection by blastoschizomyces capitatus is widely reported.[5] It has been reported to be a well-known cause of endoarditis[6], meningitis[7], osteomyelitis[8], gastrointestinal tract [9], and hepatosplenic disease.[10] Such cases were invariably among patients with severe neutropenia. B. capitatus is soil saprophyte that is a coloniser of skin and gastrointestinal tract and respiratory tract .[11] The organism was originally known as Trichosporon capitatum and then Geotrichum capitatum, but has been reclassified to the genus Blastoschizomyces.[12]

B. capitatus grows as cream coloured round to oval colonies on both blood agar and Sabouraud agar. Morphologically, similar moulds include Geotrichum candidum and Trichosporon begelli. Distinguishing features of B. capitatus include septate hyphae with narrow angle branching and the presence of conidia with tapered ends at the tip of proliferating cells, termed as anneloconidia.[13] Biochemical characteristics that help to further differentiate B. capitatus include the inability to use potassium nitrate as a sole source of nitrogen, ability to hyrolyse urea and resistant to cyclohexamide.[14] Earlier studies demonstrated fluconazole activity versus B. capitatus four to eight times greater than that of amphotericin B.[15]

CONCLUSION

This case is unique because it represents clinical disease in a patient without underlying immunosupression. Several components of the case point to B capitatus as being the infectious agent causing disease. The isolation of fungi on direct examination of repeated samples, on culture pure growth was obtained which is resuggestive of this fungus as causative agent. There is also lack of response to antibacterial therapy. In addition, clinical resolution after a course of antifungal therapy suggests significant role of B. capitatus in this respiratory tract infection.

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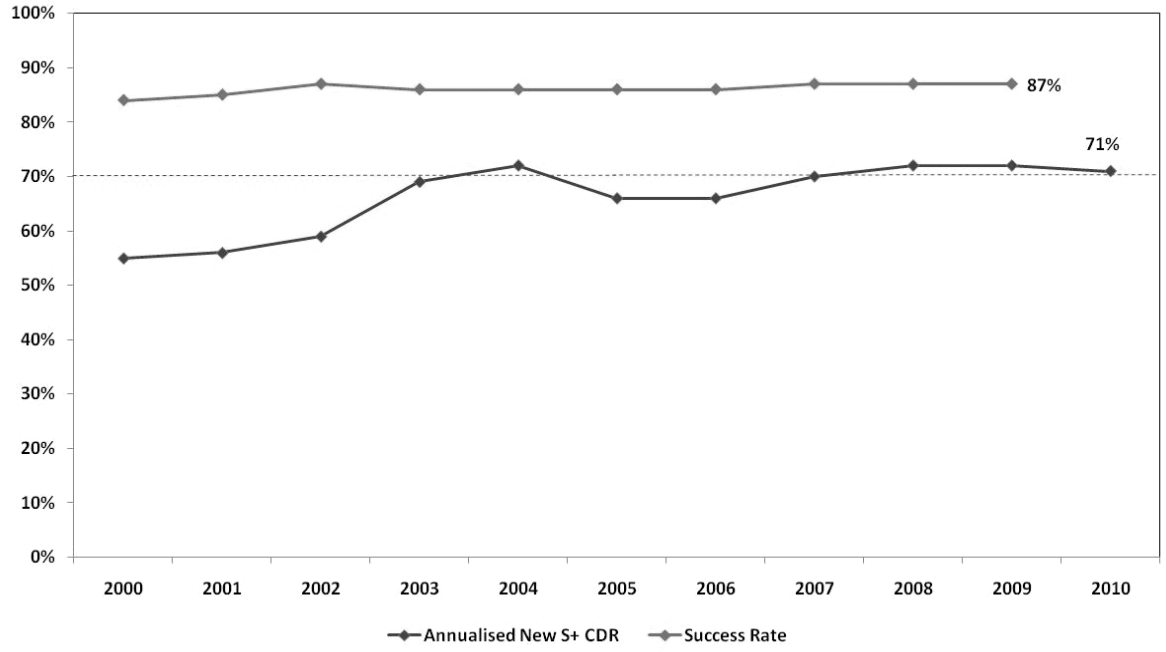
STATUS REPORT ON RNTCP*

The Revised National TB Control Programme has maintained NSP case detection rate of > 70% and treatment success rate of > 85% at the national level during the fourth quarter, 2010 (Figure 1). The programme, while consolidating and sustaining its past achievements, is progressing well towards achieving the TB related Millennium Development Goals.

RNTCP performance in fourth quarter 2010

During the quarter, over 1.84 million TB suspects were examined, 210,550 sputum positive cases were diagnosed, and 347,636 TB cases were registered for treatment. The annualized total case detection rate is 118 cases per 100,000 population. With a total of 144,974 new smear positive cases, being registered for treatment, the new smear positive TB case detection rate (annualized) for the fourth quarter 2010 is 66%. In addition to this, 82355 new smear negative cases, 50,355 new extra pulmonary cases, 46516 smear positive re-treatment cases and 22,965 re-treatment others cases were also registered for treatment during the quarter. The treatment success rate amongst the new smear positive PTB cases, registered in the fourth quarter 2009, is 88% and the sputum conversion rate of patients registered during third quarter 2010 is 90%. The default rates among NSP (5.3%), NSN (6.7%) and re-treatment cases (14.1%) continue to show the declining trend over the past several quarters.

Fig. 1: New Smear-Positive Case Detection Rate and Treatment Success Rate in DOTS areas, India, 2000-2010



•Population projected from 2001 census
•Estimated no. of NSP cases - 75/100,000 population per year (based on recent ARTI report)

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Table: Performance of RNTCP Case Detection (2010, fourth quarter), Smear Conversion (2010, third quarter), and Treatment Outcomes (2009, fourth quarter)

State	Population (in lakh) covered by RNTCP[1]	Suspects examined per lakh population	No of Smear positive patients diagnosed[2]	Total patients registered for treatment[3]	Annualized total case notification rate	New smear positive patients registered for treatment	Annualized new smear positive case detection rate (%)		New smear negative patients registered for treatment	New EP patients registered for treatment	Retreatment patients registered for treatment	3 mon conver rate new sm positi patien
Andaman & Nicobar	5	196	107	238	198	76	63	84%	62	59	41	93%
Andhra Pradesh	840	170	18347	28094	134	12238	58	78%	7057	3140	5644	92%
Arunachal Pradesh	12	201	230	472	154	149	49	65%	117	92	113	93%
Assam	302	110	4884	8855	117	3634	48	64%	2394	1172	1654	86%
Bihar	964	91	9936	17475	73	7328	30	41%	5295	1127	3574	88%
Chandigarh	14	258	473	565	165	232	68	71%	50	166	117	92%
Chhattisgarh	239	107	2942	6344	106	2394	40	50%	2364	817	750	89%
D & N Haveli	3	156	71	100	119	38	45	56%	21	18	23	92%
Daman & Diu	3	297	59	75	116	24	37	46%	26	9	16	100%
Delhi	179	205	4875	9741	217	2784	62	65%	1607	2889	2425	89%
Goa	17	234	321	536	125	183	43	53%	120	130	103	91%
Gujarat	582	193	14167	18592	128	9130	63	78%	1922	2135	5376	92%
Haryana	250	149	4731	7602	122	2704	43	46%	1437	1297	2164	90%
Himachal Pradesh	67	221	1565	2825	168	978	58	61%	480	657	701	93%
Jammu & Kashmir	116	180	2063	3050	105	1621	56	59%	386	553	486	92%
Jharkhand	310	113	4955	8729	112	4078	53	70%	2594	564	1471	92%
Karnataka	588	218	10691	17090	116	6741	46	61%	3717	3239	3387	88%
Kerala	343	265	3562	6669	78	2649	31	62%	1631	1578	808	84%
Lakshadweep	1	161	1	2	11	1	5	7%	1	0	0	75%
Madhya Pradesh	710	123	12068	20997	118	8204	46	58%	6383	2349	4036	91%
Maharashtra	1111	167	18830	33845	122	13193	47	59%	7461	5817	7374	90%
Manipur	24	141	321	798	132	240	40	53%	241	172	145	91%
Meghalaya	26	176	574	1075	166	371	57	76%	207	257	238	83%
Mizoram	10	202	138	503	203	100	40	54%	133	163	106	83%
Nagaland	22	148	449	915	165	313	56	75%	224	188	190	92%
Orissa	404	122	6517	11414	113	5022	50	59%	2756	2011	1610	88%
Puducherry	13	357	562	363	109	140	42	56%	80	85	58	89%
Punjab	274	147	5251	8453	124	3496	51	54%	1439	1653	1860	91%
Rajasthan	668	145	15351	24431	146	9323	56	70%	6477	3236	5395	92%
Sikkim	6	244	168	349	231	114	75	100%	80	78	77	83%
Tamil Nadu	670	240	10625	19663	117	7617	45	61%	5481	3703	2858	91%
Tripura	36	132	468	664	74	356	40	53%	103	107	98	88%
Uttar Pradesh	1973	137	39259	61051	124	28036	57	60%	14965	6619	11314	91%
Uttarakhand	98	167	2224	3136	128	1134	46	49%	720	498	780	89%
West Bengal	887	152	13765	22925	103	10333	47	62%	4324	3777	4489	89%

1 Projected population based on census population of 2001 is used for calculation of case detection rate. 1 lakh = 100,000 population
2 Smear positive patients diagnosed, include new smear positive cases and smear positive retreatment cases
3 Total patients registered for treatment, include new sputum smear positive cases, new smear negative cases, new extra-pulmonary cases, new others ,relapse,failure,TAD and retreatment others

Progress in accreditation of Intermediate Reference Laboratories (IRL)

Twelve IRLs across the country have already been accredited; IRL Karnal and Puducherry got accredited in this quarter. In addition, state C&DST Laboratories of UP and Uttarakhand are in the advanced stages of accreditation and the laboratories of other states are under various stages of the accreditation process. To supplement and support the state laboratory network, the programme is also involving mycobacteriology laboratories of Government Medical Colleges as well as laboratories in the NGO and Private Sector. Till date, five laboratories in other sectors (CMC Vellore, PD Hinduja Hospital Mumbai, BPHRC-Hyderabad, RMRCT Jabalpur and DFIT Nellore) have been accredited. Another five laboratories (Quest Diagnostics Gurgaon, SRL Religare, Gurgaon, SRL Religare, Mumbai, Choithtram Hospital Indore and Bhopal Memorial Hospital, Bhopal) are nearing accreditation, and several other laboratories have applied for accreditation. Apart from these, Government medical college laboratories (AIIMS New Delhi, and PGI Chandigarh) are also in the accreditation process.

Progress in the DOTS- Plus services for MDR TB cases

DOTS Plus services for management of MDR-TB are now available in 139 districts covering a population of 288 million in 12 states. Till date, a total of around 3605 MDR-TB patients are on treatment in these states. Other states are in various stages of preparatory activities for rolling out DOTS-Plus services.

Progress in TB-HIV Collaborative Activities

Intensified Package of HIV –TB collaborative activities have been rolled out in 29 states (six high prevalence states and Goa, Mizoram, Pondicherry in 2008, Delhi, Gujarat, Assam, Kerala, Punjab, Rajasthan, West Bengal, Orissa and Chandigarh in 2009 and 11 states in 2010). We would like to congratulate the states which are performing well - Karnataka, Tamil Nadu, Chandigarh, Gujarat and Goa where more than 80% of the registered TB patients in fourth quarter, 2010 know their HIV status and urge other states which are moderately performing (Maharashtra Andhra Pradesh, Mizoram, Puducharry and Delhi) and poor performing (Haryana, Assam, Kerala, Manipur and Nagaland) to accelerate progress in this regard . The proportion of HIV-positive TB patients put on CPT has improved to 93%, but linkage to ART though improved (53%) but still remains the biggest challenge.

Progress in Partnerships

The Partnership for TB Care and Control organized ACSM training for the partners in collaboration with PATH. The Partnership for TB care and control represented at the 41st World Lung conference, Berlin in November and a presentation about the added values of partnership was made. Two new members joined the partnership network in this quarter.

ABSTRACTS

The use of light-emitting diode fluorescence to diagnose mycobacterial lymphadenitis in fine-needle aspirates from children

A.C. van Wyk, B.J. Marais, R. M. Warren, S.S. van Wyk and C.A. Wright. *Int J Tuberc Lung Dis* 2011; **15(1)**: 56-60

Fine-needle aspiration biopsy (FNAB) is a simple, safe and effective method for investigating suspected mycobacterial lymphadenitis in children. Fluorescence microscopy can provide rapid mycobacterial confirmation. Light-emitting diodes (LEDs) provide a cheap and robust excitation light source, making fluorescence microscopy feasible in resource-limited settings. The objective was to compare the diagnostic performance of LED fluorescence microscopy on Papanicolaou (PAP) stained smears with the conventional Mercury Vapour Lamp (MVL). FNAB smears routinely collected from palpable lymph nodes in children with suspected mycobacterial disease were PAP-stained and evaluated by two independent microscopists using different excitatory light sources (MVL and LED). Mycobacterial culture results provided the reference standard. A manually rechargeable battery-powered LED power source was evaluated in a random subset. We evaluated 182 FNAB smears from 121 children (median age 31 months, interquartile range 10- 67). Mycobacterial cultures were positive in 84 of 121 (69%) children. The mean sensitivity with LED (mains- powered), LED (rechargeable battery-powered) and MVL was respectively 48.2%, 50.0% and 51.8% (specificity 78.4%, 86.7% and 78.4%). Inter-observer variation was similar for LED and MVL (κ = 0.5). LED fluorescence microscopy provides a reliable alternative to conventional methods and has many favourable attributes that would facilitate improved, decentralised diagnostic services.

Performance comparison of four methods for detecting multidrug-resistant *Mycobacterium tuberculosis* strains

N.M. Al-Mutairi, S. Ahmad and E. Mokaddas. *Int J Tuberc Lung Dis* 2011;**15(1)**: 110-5

The study was conducted at National Tuberculosis Reference Laboratory, Kuwait. The objective was to compare Genotype *MTBDR plus* (gMTBDR$^+$), INNO-LiPA Rif. TB (INNO-LiPA), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and DNA sequencing for detecting rifampicin (RMP) and/or isoniazid (INH) resistance-associated mutations in the rpoB hot-spot region (HSR- *rpoB),* the *katG* codon 315 *(katG315)* and the *inhA* regulatory region *(inhA-RR)* among multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) isolates. A total of 82 MDR- TB and 43 pansusceptible *M. tuberculosis* BACTEC 460-characterised isolates were processed using molecular techniques and the Mycobacterial Growth Indicator Tube (MGIT) 960 system. All susceptible strains contained wild-type sequences in target genes. RMP resistance was detected in respectively 78, 77 and 79 MDR- TB strains by gMTBDR$^+$, INNO-LiPA and HSR-rpoB sequencing. Two isolates with Ins514TTC mutation were detected as RMP-resistant by gMTBDR$^+$ but as RMP-susceptible by INNO-LiPA. One isolate with L533P mutation, detected as RMP- susceptible by gMTBDR$^+$, was detected as RMP-resistant by INNO-LiPA. Two of three isolates detected as RMP- susceptible by gMTBDR$^+$, INNO-LiPA, HSR-rpoB sequencing and the MGIT 960 system contained a I572F mutation that is outside HSR-rpoB. INH resistance was detected in respectively 76, 60, 60 and 22 MDR- TB strains by gMTBDR$^+$, *katG315* PCR-RFLP, *katG315* sequencing and *inhA-RR* sequencing. Although gMTBDR$^+$ accurately detected ~ 88 % of MDR-TB strains, some *rpoB* mutations were either missed or were outside the region of analysis of the gMTBDR$^+$ assay.

Screening for tuberculosis in asylum seekers: comparison of chest radiography with an interview-based system

S.Schneeberger Geisler, P. Helbling, J.P. Zelfweger and E.S. Altpeter *Int. J. Tuberc Lung Dis* 2010; **14(11)**: 1388-94

It was a mandatory initial screening of asylum seekers for tuberculosis (TB) in Switzerland, 2004-2005 and 2007-2008. The objective was to compare the yield of screening by chest radiography with an individual assessment based on geographic origin, personal history and symptoms. It was a cross-sectional retrospective comparison of two 2-year periods. The prevalence of detected TB cases was defined as the proportion of screenees starting anti-tuberculosis treatment for culture-confirmed pulmonary TB within 90 days. TB prevalence was 14.3 per 10000 asylum seekers screened (31/21727) using chest radiography and 12.4 (29/23402) using individual assessment. The sensitivity of radiography was 100% vs. 55% for individual assessment, but its specificity was lower (89.9% vs. 96.0%, respectively). The higher sensitivity of radiography meant shorter delays between screening and start of treatment (median 6 vs. 25 days). Its lower specificity led to a larger proportion of screenees needing further investigations for suspicion of TB (12% vs. 4%). The interview-based system initially missed more cases, but the ultimate 90-day yield was comparable for the two periods. The main difference is the delay until start of treatment, which potentially increases transmission and secondary cases. The radiograph system was more burdensome to both the health care system and the screenees, as more suspects required further investigations.

Concomitant increases in spectrum and level of drug resistance in *Mycobacterium tuberculosis* isolates

Z. Sun, J. Zhang, H. Song, X. Zhang, Y. Li, M. Tian, Y. Liu, Y. Zhao and C. Li. *Int J Tuberc Lung Dis* 2010; **14(11)**: 1436-41

The objective was to determine the drug resistance spectrum and resistance levels of Extensively Drug-Resistant (XDR-) and Multi Drug-Resistant Tuberculosis (MDR-TB) and TB resistant to either rifampicin (RMP, R) or isoniazid (INH, H; R/H-DR). Of 142 drug-resistant clinical isolates examined, 13 were XDR-TB, 66 were MDR-TB and 63 were R/H-DR. The drug resistance spectrum was tested by the absolute two-concentration method. Minimum Inhibitory Concentrations (MICs) were determined for the strains by agar dilution method on Lowenstein-Jensen slants. The drug resistance spectrum of XDR-TB, MDR-TB and R/H-DR TB isolates ranged from 4 to 9, 2 to 6 and 1 to 5 drugs, respectively. Over half of all XDR-TB (53.8%), MDR-TB (66.7%) and R/H-DR (54.0%) isolates were resistant to two other anti-tuberculosis drugs; 38.5% of XDR-TB, 24.2% of MDR-TB and 28.6% of R/H-DR TB isolates were resistant to $\geq$ 3 additional anti-tuberculosis drugs in addition to those originally defined, demonstrating that the MIC values and the proportions of strains with higher MICs followed a trend of XDR-TB > MDR- TB > R/H-DR for INH, RMP, ofloxacin and ethambutol. XDR-TB, MDR-TB and RlH-DR TB isolates exhibited both increasingly broader resistance spectra and a higher percentage of strains with high MICs to more frequently resistant drugs, which might be related to patterns of TB chemotherapy.

Role of whole-blood interferon-gamma release assay in the diagnosis of smear-negative tuberculosis

E.C.C. Leung, C.C. Leung, W.W.L. Leung, K.M. Kam, W.W. Yew, S.N. Lee and C.M. Tam. *Int J Tuberc Lung Dis* 2010; **14(12)**: 1564-70

The study was conducted at Hong Kong Chest Clinics. The objectives and methods were to conduct a prospective study investigating the role of a whole-blood interferon- gamma release assay, QuantiFERON-TB® Gold In-Tube (QFT-GIT), in the diagnosis of smear-negative tuberculosis (TB). The QFT-GIT result was compared with the final confirmed diagnosis after 12 months. Of 262 smear-negative subjects, 188 had active TB, 167 (88.8%) of whom were QFT-GIT-positive; 74 had inactive/non-TB, 30 (40.5%) of whom were QFT-GIT-negative. The positive (PPV) and negative predictive values for active

TB were respectively 79.1 % and 58.8%. For this target group with high TB prevalence (71.8%), a positive test increased the chance of active disease by only 7.3 %. Despite a positive Likelihood Ratio (LR) of 1.4:9, the negative LR was 0.28, making the diagnosis of active TB much less likely after a negative test. Although sensitivity and specificity showed no difference across different age groups, the PPV decreased ($P < 0.001$) with increasing age, likely reflecting the increased prevalence of competing diagnoses. In an area with a high prevalence of latent TB infection, a positive QFT -GIT test does not add much to confirm the diagnosis of smear-negative TB, while a negative test indicates a need for further investigation.

Tuberculosis among community-based health care researchers

M.M. Claassens, C. Sismanidis, K.A. Lawrence, P. Godfre-Faussett, H. Ayles, D.A. Enarson and N. Beyers. *Int J Tuberc Lung Dis* 2010; **14(12)**: 1576-81

Occupational tuberculosis (TB) in hospital-based health care workers is reported regularly, but TB in community-based health care researchers has not often been addressed. The objective was to investigate TB incidence in health care researchers in a high TB and human immunodeficiency virus prevalent setting in the Western Cape, South Africa. The health care researchers were employed at the Desmond Tutu TB Centre, Stellenbosch University. A retrospective analysis was performed of routine information concerning employees at the Desmond Tutu TB Centre. The Centre has office-based and community-based employees. Of 180 researchers included in the analysis, 11 TB cases were identified over 250.4 person-years (py) of follow-up. All cases were identified among community- based researchers. TB incidence was 4.39 per 100 py (95%CI 2.45-7.93). The standardised TB morbidity ratio was 2.47 (95%CI 1.25-4.32), which exceeded the standard population rate by 147%. TB incidence in South Africa was 948 per 100000 population per year in 2007; in the communities where the researchers worked, it was 1875/100000. Community-based researchers in the study population have a 2.34 times higher TB incidence than the community. It is the responsibility of principal investigators to implement occupational health and infection control guidelines to protect researchers.

Is the T-Cell-Based Interferon-Gamma Releasing Assay Feasible for Diagnosis of Latent Tuberculosis Infection in an Intermediate Tuberculosis-Burden Country?

Asli Gorek Dilektasli, Elif Erdem, Elif Durukan and Fusun Oner Eyuboglu. *Jpn J Infect Dis* 2010; **63:** 433-6

The diagnosis of active and latent tuberculosis infection (L TBI) remains a challenge, especially in light of the fact that the Tuberculin Skin Test (TST), which has been used to diagnose L TBI for over a century, has many well-known drawbacks. This study aimed to compare the diagnostic performance of the T-cell-based interferon-y releasing assay (IGRA) T -SPOT. TB with the TST for the diagnosis of L TBI in an intermediate tuberculosis (TB)-burden country with high BCG coverage. For this purpose, a total of 91 participants, including culture-confirmed TB patients, healthy contacts known to have been exposed to *Mycobacterium tuberculosis,* and healthy volunteers, selected from a BCG-vaccinated population were recruited. The sensitivities of the T-SPOT. TB and TST were 79.3 and 25.8%, and the specificities were 75.9 and 56.7%, respectively. The negative- and positive-predictive values for T-SPOT. TB and TST were 78.6 and 76.7% and 42.5 and 38.1%, respectively. The diagnostic performance of the TST in L TBI diagnosis is therefore severely diminished in BCG-vaccinated populations, with the sensitivity and specificity of the T-SPOT. TB assay being markedly higher. IGRAs have been reported to have higher diagnostic sensitivity and specificity in low TB-incidence settings than those seen here. Further larger scale studies in high and intermediate TB-incidence settings are therefore needed.

Obituary

Dr. S.N. Tripathy
(1938-2011)

Dr. S.N. Tripathy, Chairman, National College of Chest Physicians (East Zone) passed away on 14.3.2011 at Cuttack.

The Tuberculosis Association of India places on record its deep sense of sorrow and profound grief on the loss of this anti-TB stalwart.

Born on 12.9.1938 in Muraripur, Cuttack district, Dr. Tripathy did his M.B.B.S. from the Utkal University in 1962 and TDD and MD in Pulmonary Medicine from the Grant Medical College, Bombay in 1965 and 1966 respectively. He was Fellow of National College of Chest Physicians (FNCCP).

He served in various capacities in the Medical Colleges of Orissa retiring as Professor and Head, Department of Pulmonary Medicine, SCB Medical College, Cuttack and Superintendent, Anti-TB Demonstration Centre, Cuttack.

A teacher par excellence and a postgraduate examiner to various universities, he was the recipient of numerous honours and awards, including that of the Tuberculosis Association of India for his outstanding contributions in the field of tuberculosis. He was the President of Annual National Conference of Tuberculosis Association of India held at Goa in 2000 and Chairman of the Standing Technical Committee the same year.

Dr. Tripathy conducted many a basic research in the field of tuberculosis and chest diseases. His research in genito-urinary tuberculosis earned him world-wide recognition. He had to his credit various national and international publications and was the Editor of the book on Genital Tuberculosis. He had been closely associated with many community welfare organizations in various capacities for over four decades.

A man of unusual conviction and clinical skills, his broad knowledge of pulmonary medicine and tuberculosis, played a vital role in creating and evolutionising the Department of Pulmonary Medicine in VSS Medical College, Burla, and also brought The Department of Pulmonary Medicine of SCB Medical College to the forefront.

Being a distinguished investigator, teacher and academician, he was first and foremost a masterful physician and a wonderful human being who excelled in the care of the sick. His method of diagnosis and treatment and the empathetic attitude towards patients was legendary. He was the man who initiated and brought RNTPC to Odisha.

The demise of Dr. Tripathy has created a void which is difficult to fill. The Tuberculosis Association of India mourns his loss and conveys its deep condolences to the bereaved family. May God grant eternal peace to the departed soul is our prayer.
