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Editorial

IMMUNODIAGNOSTICS FOR TUBERCULOSIS - PROBLEMS AND PROGRESS

[Indian J Tuberc 2010; 57:123-127]

Tuberculosis (TB) is the seventh leading cause of death among infectious diseases. Sputum smear microscopy has remained the corner stone of TB diagnosis in the global strategy to control the disease[1]. The global targets for TB control, adopted by World Health Assembly, are to cure 85% of the newly detected smear positive TB cases and to detect 70% of the estimated incidence of sputum smear positive TB cases[2]. 70% of case detection still leaves behind a gap of 30% of cases yet to be detected. Further, the sensitivity of sputum smear microscopy is limited requiring >10000 bacilli per ml of sputum and not useful in extra pulmonary TB and paediatric tuberculosis. Culture is the most sensitive method for detecting TB, but it can take several weeks to yield results and demands advanced technical infrastructure that is not widely accessible in resource - limited health systems. A novel sample processing methodology (Universal sample processing methodology, USP) was developed by Chakravorty and Tyagi[3]. Specimens containing ~300 to 400 bacilli per ml can be reproducibly detected as positive by USP smear microscopy. Automated Liquid Culture Systems such as BACTEC and MGIT reduce the delays in obtaining results to days rather than weeks. However, they are expensive and require expertise and infrastructure. Improvements have been made in culture techniques. Bhattacharya *et al*[4] developed a relatively rapid, low cost and safe bilayered medium with tetrazolium indicator achieving higher isolation rates. Performance of a filtration step was shown to improve the culture yield from paucibacillary body fluids including cerebrospinal fluid (CSF)[5]. Singh *et al*[6] have described various methods for improving the performance of commercial liquid culture techniques in the Indian scenario.

In areas heavily burdened by HIV, due to failure of lab tests in detecting TB, observing response to anti-tuberculosis therapy has been evaluated as diagnostic strategy to identify TB in HIV-TB coninfection[7]. Recently, two rapid tests that use nucleic acid amplification (NAA) have been approved by US FDA for the diagnosis of TB, were evaluated in a large urban setting with moderate TB prevalence and observed a sensitivity of 96% and specificity of 95% in specimens with positive AFB smear while sensitivity of 79% and specificity of 80% in TB specimens tested negative for AFB on smear[8]. However NAA test requires sophisticated facility and infrastructure and is not accessible in district hospital setting in developing countries.

The involvement of agencies such as Stop TB Partnership's working group on new diagnostics, WHO, TDR and the Foundation for Innovative New Diagnostics (FIND) has led to a resurgence of interest in the development of new TB diagnostics.

TB serology based on antibody and antigen detection for diagnosis and monitoring tubercular infection with low cost and flexibility to adapt to field laboratories may be a boon to developing countries. Immunodiagnostic tests are relatively simple to use, inexpensive and easy to interpret and also better for detecting extra pulmonary tuberculosis and for TB in children. Presence of antibody to an infectious agent is an early immune response of infected host and thus is an early indicator of infection. Antigen assay will be a better marker for presence of bacilli and correlates with bacterial burden. Two recent systematic

reviews on immunodiagnostics for PTB and EPTB have analysed the available evidence on serological tests for TB and came to the conclusion that none of the assays performed well enough to replace smear microscopy[9,10]. The possible reasons of failure in immunodiagnosis are (1) Mostly single antigen based antibody assays are explored and introduced in the form of a kit without thorough evaluation in an hospital setting and (2) Further, there are a few single or cocktail antigen/IC antigen detection assays reported in literature.

Diagnosis of latent TB infection (LTBI) by T-cell based interferon – gamma release assays (IGRA) has been the biggest advance in recent times as a substitute to tuberculin skin test (TST). This is an *in vitro* blood test based on interferon – gamma release after stimulation by TB specific antigen (eg. ESAT-6 and CFP-10). Two IGRAs are now commercially available – the Quantiferon – TB Gold in – Tube assay and the T-SPOT TB assay. In a field study on IGRA vs TST, no significant difference was observed between the two in detection of latent infection[11]. ES-6 (6 kDa) antigen has shown increased seroreactivity in household contacts of TB cases[12].

In an encouraging recent study, Anderson *et al*[13] evaluated three commercially available serologic assays for detection of *M.tb* antibodies in patients (US born individuals) with active disease namely InBios Active TB detect IgG, ELISA; IBL *M.tb* IgG ELISA and Anda Biologics TB ELISA and observed an agreement of 96.2%, a sensitivity of 83.3% and specificity of 98.9% with InBios Active TB Detect ELISA and was found to be quite superior to the other kits. InBios test kit utilizes several antigens including *M.tb*81, *M.tb*8, *M.tb*48, DPEP (MPT32), 38 kDa protein and two additional proprietary antigens. This needs further evaluation in countries with increased incidence of TB. Urine antigen testing is an attractive strategy for the diagnosis of active tuberculosis. Based on the excretion of LAM antigen in urine, a commercial kit marketed as Clearview® TB ELISA was evaluated in culture positive TB cases and observed low sensitivity and not suitable in the current format[14].

Most of the immunodiagnostic assay systems which work satisfactorily in tailored protocols are not working in the field to the satisfaction of the clinicians. Well researched and promoted LAM based test kits are an example of failure. A thorough study is needed in an hospital setting to understand the diagnostic advantages and problems (false positivity and false negativity) in sputum positive, sputum negative TB and in particular clinically suspected and smear negative cases of PTB and EPTB to make the assay system acceptable as second best after sputum smear or culture. An ELISA for EPTB is more convenient and will be accessible compared to FNAC or biopsies in rural hospitals.

In spite of a large number of studies for number of years throughout the world, why serology failed? It is important to understand the reasons for better progress. Tuberculosis infection is a complex one. PTB is seen as latent, fresh, chronic, relapse and resistant and EPTB affecting other organs. Different antigens have shown variable immune response namely ES-31 in chronic PTB cases, ES-43 in relapse cases, ES-41 in bone and joint TB which should be utilized for improved diagnosis[12,15,16]. Further extra pulmonary TB with paucibacilli is difficult to diagnose. Immune response depends on the interaction between the pathogen and the infected host where the latter may be weak immune, strong immune influencing the antibody response and antigen clearance. Elevated ES-20 antigen levels were observed in weak immune patients of TB lymphadenitis[17]. Even though antigen is better marker, antigen clearance by the infected individual may also affect its levels. Presence of immune complexed antigen may also help in confirming the disease. Detection of antigen and immune complexed antigen using anti ES-31 antibody increased the sensitivity of the immunoassay[18]. Antigen assay was found to be more sensitive than antibody assay in immunocompromised HIV infected sera[19]. Another reason for failure of serology, is expectation that it should be similar to sputum smear or culture, the gold standard, in spite of limitations of its

sensitivity and decreased use in hospital setting for time constraint and delay in diagnosis. Lastly, the reason for failure is lack of thorough evaluation in hospital setting. Further, greediness of commercial manufacturers is responsible to push the test and make a fast buck. IgG and in particular IgG_1 and IgG_3 were found to be more useful for detection of TB[20]. Usually a commercial firm introduces kits for anti TB IgG, IgM and IgA antibody simultaneously but without detailed study and exposition on their utility, if any, in specific cases of tuberculosis. As a result, the tests are not helpful in coming to a definite conclusion. Thus the commercial kits could not stand for scrutiny. IgG subtype, antigen and immune complexed antigen assays may help in improved detection of TB. Further antigen assay was found to be useful in determining therapeutic effectivity and compliance which is an important problem in successful management of TB[12].

Mukherjee *et al*[21], have explored the serodiagnostic potential of a panel of RD1 antigens and the culture filtrate proteins for the diagnosis of PTB and EPTB. Among the antigens Rv3872, Rv3878, ESAT-6, CFP-10, CFP-11, CFP-31, Ag85A and Ag85B tested, Rv3872 and its antigenic epitope (amino acids 57 to 84) emerged as promising antigens.

In this context constant evaluation of two in house developed ELISA assay systems for PTB and EPTB in hospital setting are of interest.

Kashyap *et al*[22] reported ELISA for detection of Ag85 complex (30kDa protein) in CSF samples of Tuberculous Meningitis (TBM) with a sensitivity and specificity of 89% and 92%. In addition they have reported good sensitivity and specificity with Hsp 65 antigen in developed ELISA system for TB and TBM diagnosis[23].

The mycobacterial excretory secretory (ES) proteins released in the culture medium have been of considerable diagnostic interest. Excretory secretory (ES) proteins of *Mycobacterium tuberculosis (M.tb.)* such as ES-31, ES-41, ES-43, ES-6, ES-20 and EST-6, isolated from *M. tb.* H_{37}Ra culture filtrate have been explored.

A cocktail of antigens ES-31, ES-43 and ES-41 detecting antibody showed improved sensitivity (96%) in PTB cases[24]. A cocktail of affinity purified antibodies to antigens ES-31, ES-43 and EST-6 (ES-41+ES-38) showed a sensitivity of 91% for detection of antigen and 97% for IC-Ag with specificity of 95% and 99% respectively. Similar observations were made for detection of antibody and antigen in patients of extrapulmonary tuberculosis[25, 26]. Detection of antibody, circulating free and immune complexed antigen is done for patients on the request of the clinicians. In a prospective study, the in house developed SEVA TB ELISA analyzed for nine months in tuberculosis suspected patients showed 100% correlation (42 cases) with AFB positivity or ATT treatment. However, 36% of clinically suspected cases showed ELISA positivity but were not ATT treated. These cases need to be followed up for development of disease or possibly treated as latent infection. The study further showed importance of ELISA with positivity in AFB negative TB but clinically diagnosed and ATT treated cases[27]. This can make an important contribution in confirming TB and correlating with clinical decision in smear negative TB cases in PTB and EPTB.

In the diagnosis not only we come across pathogen, immune status of infected host but we also face the clinicians with his/her clinical judgment of the case adding to the complexity of diagnosis. Our approach to immunodiagnostics cannot be mathematical in terms of percentage sensitivity and specificity but needs to be pragmatic to make it useful to the clinicians in diagnosis, monitoring and management of patients for continued patronage. It is hoped that judicial use of cocktails of antigens, assays of antibody,

free and immune complexed antigens with evaluation in an hospital setting should help in developing a successful immunodiagnostic test profile for tuberculosis.

B.C. Harinath
Director
JB Tropical Disease Research Centre
Mahatma Gandhi Institute of Medical Sciences
Sevagram (Wardha) – 442 102
e-mail: bch@jbtdrc.org, bc_harinath@yahoo.com

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Original Article

EVALUATION OF POLYMERASE CHAIN REACTION (PCR) USING *hupB* GENE IN DIAGNOSIS OF TUBERCULOUS LYMPHADENITIS IN FINE NEEDLE ASPIRATES

Pooja Verma, Anju Jain[1]*, Surajeet Kumar Patra, Shipra Gandhi[2], B.L. Sherwal[1] and Monisha Chaudhary[1]*****

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Summary

***Backround*:** Although pulmonary tuberculosis(PTB) is the most common manifestation of tuberculosis, extra pulmonary tuberculosis(EPTB) has equal significance. Among the extra pulmonary manifestations, tubercular lymphadenitis (TBL) is the most common form.

***Objectives*:** To perform PCR on fine needle aspirates of lymphnode by using *hupB* gene as target. To compare the sensitivity and specificity of PCR with culture, cytology, serology and clinical response to therapy.

***Material & Methods*:** After processing the samples by Universal Sample Processing(USP) method,two step nested PCR was performed using two sets of primers(N1S1 & CTFR) of *hupB gene*. All patients were put on ATT and were followed up for two months. The response to therapy was considered as the gold standard in our study.

***Results*:** The PCR assay for *hupB* gene was positive in 85 patients. Of these, 82% patients showed infection with *M. tuberculosis*, 1% was positive for *M. bovis* and 2% showed co- infection with both *M. tuberculosis* and *M. bovis.* The PCR assay of *hupB* gene in our study showed a sensitivity of 87.4% and specificity of 66.7%.

Conclusion: PCR assay for *hup B* gene is a rapid means of diagnosis of tubercular lymphadenitis.

[Indian J Tuberc 2010; 57:128-133]

Key words: Tubercular lymphadenitis, *hupB* gene.

INTRODUCTION

Tuberculosis(TB) is the leading cause of mortality in adults due to an infectious agent. There were an estimated 1.9 million new cases of TB occurring annually and out of which around 0.8 million have sputum smear positive pulmonary tuberculosis[1]. The global annual incidence of tuberculosis is 9.1 million and Indian annual incidence is 1.9 million[2]. India is the highest TB burden country globally, accounting for one fifth of the global incidence [2].

Although pulmonary tuberculosis (PTB) is the most common manifestation of tuberculosis, extra pulmonary tuberculosis (EPTB) has equal significance. Among the extra pulmonary manifestations, tubercular lymphadenitis (TBL) is the most common form. TBL also occurs with an increased frequency in patients with HIV[3].

The Revised National Tuberculosis Control Programme (RNTCP) diagnostic algorithm for TBL includes lymph node enlargement of > 2 cm in one or more sites, should be prescribed a course of antibiotics for two weeks and if lymph node enlargement still persists, then we can suspect tubercular lymphadenitis. This diagnosis is confirmed if Fine Needle Aspiration Cytology (FNAC) shows granulomatous changes and/or Ziehl-Neelsen stain positive for AFB.

The conventional methods for the diagnosis of TB like AFB smear microscopy are useful but are limiting in TBL. Culture is considered as "gold standard" for diagnosis but has limitation in terms of time (4-6 weeks) in EPTB. Thus recently focus has shifted towards molecular diagnostic methods[4] but these are yet to be included in diagnostic algorithm of any RNTCP programme.

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1. Professor 2. Short term research student for ICMR project

Departments of Biochemistry*, Microbiology** and Pathology***, Lady Hardinge Medical College and Associated Hospitals, New Delhi.

***Correspondence*:** Dr. Anju Jain, Professor, Department of Biochemistry, Lady Hardinge Medical College and Associated Hospitals, New Delhi-110 001; M - (+91) 9811519290; E-mail: dranjujain@rediffmail.com; drsurajeetkumarpatra@rediffmail.com

Of the various molecular methods available, amplification of *hup B* gene *(RV 2986 C)* using PCR is of special significance as it can detect and differentiate between *M. tuberculosis* and *M. bovis* [5-7].

MATERIAL AND METHODS

The study was conducted jointly in the Departments of Biochemistry, Microbiology and Pathology of Lady Hardinge Medical College and Smt Sucheta Kriplani Hospital New Delhi. The study was approved by the Ethical Committee of Lady Hardinge Medical college, New Delhi. One hundred consecutive cases were enrolled in our study after informed consent. Fine needle aspirates were taken from patients coming to Department of Pathology, Lady Hardinge Medical College, New Delhi.

Selection criteria

Patients of any age or sex presenting with history of palpable lymph node (>2cm) at any superficial site of the body, not relieved by two weeks of antibiotic course and fine needle aspirate showing a tuberculous lesion on cytology and/or identification of bacilli on Ziehl-Neelsen (ZN) stain[9]. This is as per the diagnostic algorithm for TBL in RNTCP[8,10,11].

Exclusion criteria

History of known malignancy/Human Immunodeficiency Virus infection/Diabetes mellitus/ Immunosuppressive state or therapy, FNAC showing reactive or any other cause of lymphadenopathy.

A detailed history and clinical examination was done for all patients. Routine laboratory tests like complete hemogram with Erythrocyte sedimentation rate, Liver Function Tests(Serum bilirubin, Alanine aminotransferase, Aspartate aminotransferase),Kidney Function Tests(Blood urea,serum creatinine, serum uric acid), Mantoux test and X-ray chest were done for all patients. Serum samples were also collected from patients for estimating antibodies against 38kDa mycobacterial antigen by Enzyme Linked Immuno Sorbent Assay (ELISA) using Pathozyme TB plus kit from Omega Diagnostics Limited, UK.

Fine-needle aspirates

Fine-needle aspirates from the involved lymph node were divided into four aliquots. Smears were air-dried and stained for Ziehl-Neelsen and Giemsa stains. Others were used for culture on Lowenstein-Jensen (LJ) medium and for PCR.

Sample processing

Samples were processed by Universal Sample Processing(USP) method[14] which involved homogenization and decontamination of the specimens by treatment with USP solution (4 to 6 M guanidinium hydrochloride, 50 mM Tris-Cl, pH 7.5, 25 mM EDTA, 0.5% Sarcosyl, 0.1 to 0.2 M â-mercaptoethanol)[14].

PCR

Two step nested PCR was performed using two sets of primers of *hupB* gene[13, 17].

N- 5'-GAG GGT TGG GAT GAA CAA AGC AG-3'
S- 5'-TAT CCG TGT GTC TTG ACC TAT TTG-3'
CTF 5'-CCA AGA AGG CGA CAA AGG-3'
CTR 5'-TTA GGG GAC ACC AAG CCC TCA GGA AGA GCA-3'

The first amplification of *hup B* gene was done by using 40ì l reaction mixture (1X buffer, 12.5mM $MgCl_2$, 200 ì M of dNTPs (CTP, TTP, GTP, ATP), 0.5ì l each of forward and reverse primers, 1U Taq Polymerase.). N1S1 PCR consists of 95^0 ×10 minutes(min), 94^0 ×1.30 min, 60^0×1.30 min, 72^0×1.50 min, and 72^0 ×30 min. A total of 35 cycles were used. This amplified the whole *hup B* gene of 645 bp and 618bp in *M.tuberculosis* and *M. bovis* respectively. This amplicon was again subjected to PCR using CTF and CTR primers. CTFR PCR consists of 95^0 ×10 min, 94^0 ×1min ,60^0 × 0.30 min, 72^0×7 min. The C terminal forward and reverse primers amplified the C terminal of *hup B* gene to identify 27 bp deletion between *M.tuberculosis* and *M. bovis.* The amplicons obtained after the second amplification were 118 bp and 89 bp for *M.tuberculosis* and *M. bovis* respectively.

The final products were then resolved in 8% Poly Acrylamide Gel Electrophoresis(PAGE) and viewed under gel documentation system (Figure).

Follow up

All patients were put on ATT and were followed up for two months. The response to therapy was considered as the gold standard in our study.

Statistical analysis

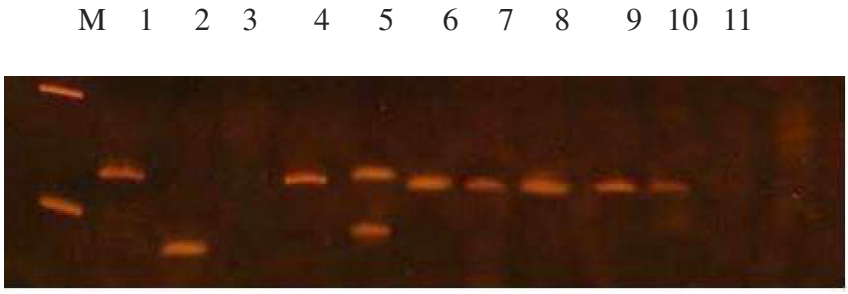
Taking response to therapy as gold standard, sensitivity and specificity of different assay methods

Table 1: Clinico-epidemiological characteristics of patients with Tubercular lymphadenopathy(N=100)

Clinical parameters		Site of Lymphadenopathy	
		Cervical	78
Mean age	21.7±9.9	Axillary	10
Female : Male	1:0.69	Submandibular	6
History of contact	34	Supraclavicular	4
Past history of TB	5	Inguinal	2
Fever	48		
Anorexia	46		
Weight loss	28		

Table 2: Type of infection by *hup B* gene assay

Type of infection	No. of patients
M.tuberculosis	82
M.bovis	1
Co-infection	2



M: 100 bp ladder
Lane 1: *M. tuberculosis* positive control
Lane 2: M. bovis positive control
Lane 3: Negative control
Lane 4, 6,7,8,9,10: Lymphnode samples positive for *M. tuberculosis*
Lane 5: Coinfection of *M. bovis* and *M. tuberculosis*
Lane 11: Negative lymphnode samples

Fig: Nested PCR products for *hupB* gene using primers CTF/R resolved on 8% Polyacrylamide gel

Table 3: Comparative analysis of microbiological and serological assays with response to therapy (n = 98*)

Investigation	ATT Responsive	ATT non responsive	Sensitivity (%)	Specificity (%)	Positive Predictive value (%)	Negative Predictive Value (%)	p Value
Serology	45	0	47.4	100	100	5.7	0.600
Culture	8	0	8.4	100	100	3.3	0.05
PCR assay	85	1	87.4	66.7	98.8	14.3	0.008

*2 patients could not be followed up

were calculated. The results were analyzed with SPSS version 12.

RESULTS

In our study, we observed that 83% of the patients were in the age group of 10-30 years with mean age of 21.7 ± 9.9. Of the total patients, 59% were females and 41% were males (Table 1).

In 8% cases, positive culture was seen. The antibodies against 38kDa antigen by ELISA were positive in 45% of patients only. Out of 98 patients who could be followed up, response to ATT (Category I,2HRZE+4HR)was seen in 95 patients.

In our study, it was observed that 85% of the patients had positive PCR assay for *hupB* gene. Out of these, 82% of the patients showed *M. tuberculosis* infection, only 1% was infected by *M. bovis* and 2% had co-infection with both *M. tuberculosis* and *M. bovis* (Table 2).

Culture was positive in only eight cases thereby showing a sensitivity of 8.4%. Antibody assay also showed a sensitivity of 47.4%. PCR assay showed a sensitivity and specificity of 87.4% and 66.7% respectively (Table 3).

DISCUSSION

Of the hundred patients cytologically diagnosed as tuberculous/granulomatous lymphadenitis, 83 were in the age group of 10-30 years with mean age of 21.7 ± 9.9. 59% of the patients were females with female: male ratio of 1:0.69. A similar observation was reported in a study by Pahwa *et al*[3] where the median age of the patient was 21.5 and female: male ratio of 1:0.75[3].

It has been documented that although there is a high probability of being infected following prolonged contact with an infectious source, persons infected outside the context of close contact may be high[12]. In our study, 34% of the patients had a history of contact.

X-ray chest showed concomitant old healed lesions in 17% of patients. This indicates that chest X-ray cannot be used as a diagnostic parameter for tuberculous lymphadenitis *per se* unlike the pulmonary form of the disease.

Adjunctive test, which is usually carried out, is the Mantoux skin test. Our study showed a positive skin test in 55% of the patients (n=100). Low reactivity (<10mm) is seen in the rest of the patients. However, interpretation of this test should be carried out with caution as a positive result does not always imply a diseased state.

Among the patients presented with superficial lymphadenopathy, 78% of the patients had cervical lymphnode enlargement and 10% showed involvement of axillary lymphnodes (Table 1). This is in accordance with a study by Bem *et al*[11] who observed that cervical lymph nodes were

most commonly affected followed by axillary lymph nodes.

The presentation of EPTB is often non-specific and insidious unlike pulmonary tuberculosis where the patients are usually symptomatic[15]. Fever was the most common presenting complaint of the patients along with anorexia. 48% presented with low grade fever and 46% complained of loss of appetite.

When serum was analysed for antibodies against 38kDa mycobacterial antigen, they were raised in 45 cases, thereby showing a sensitivity and specificity of 47.4% and 100% respectively (Table 3).

The PCR assay for *hupB* gene and its further amplification of the C-terminal was positive in 85 patients. Of these, 82% patients showed infection with *M. tuberculosis*, 1% was positive for *M. bovis* and 2% showed co-infection with both *M. tuberculosis* and *M. bovis* (Table 3).

The PCR assay of hup*B gene* in our study showed a sensitivity of 87.4% and specificity of 66.7% (Table 3). This is in agreement with previous studies on the same gene target in EPTB. Studies on various biological samples like CSF, ascitic fluid, endometrial biopsies conducted in our laboratory have shown good sensitivity and specificity of PCR assay targeting *hupB* gene. A study by Shah *et al* on extra pulmonary CSF samples using the same gene target showed sensitivity and specificity of 60% and 88.6% respectively[16]. Another study on TBM utilizing the target showed a sensitivity of 92% and specificity of 98.7%[17]. The results of this assay using this gene target as a diagnostic tool for tuberculous lymphadenitis are highly significant (p=0.008).

The PCR assay for *hupB* gene used in this study could fulfill two conditions- a rapid means of diagnosis of tuberculosis as well as a tool for differentiation between *M. tuberculosis* and *M. bovis* and is useful in conditions where the conventional diagnostic methods are limiting. Thus, we recommend the inclusion of molecular methods in diagnostic algorithm in EPTB as part of RNTCP programme.

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The Annual General and Central Committee meetings of the Tuberculosis Association of India were held on 29th May, 2010. Both the meetings were presided over by Dr.S.P Agarwal, President of the Association.

Dr. V.K. Arora, Vice-Chairman, TAI welcomed those present at the Annual General Meeting. Dr. R.K. Srivastava, Chairman, presented the Annual Report for 2007-2008 & 2008-2009 and Shri M.P. Gupta, Honorary Treasurer, presented the audited accounts of the Association for both the years. The President and Chairman gave away the annual awards.

Original Article

SMEAR MICROSCOPY AS SURROGATE FOR CULTURE DURING FOLLOW UP OF PULMONARY MDR-TB PATIENTS ON DOTS PLUS TREATMENT

R. Sarin[1], R. Singla[2], P. Visalakshi[3], A. Jaiswal[4], M.M. Puri[4], Khalid U K[5], K. Mathuria[6], N. Singla[7], D. Behera[8] and P.P. Sharma[9]

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Summary

Background: DOTS Plus site at LRS Institute, New Delhi, covering 1.8 million population.

***Aims*:** To ascertain if sputum smear could be used as a surrogate for culture during intensive phase of treatment of MDR-TB patients thereby enabling early shift from intensive phase to continuation phase, reducing the need for frequent cultures and saving time and cost in their management.

***Methods*:** The study is a retrospective analysis of 138 MDR-TB patients on DOTS Plus treatment whose sputum samples were simultaneously subjected to smear microscopy and culture, monthly during Intensive Phase and once in two months during Continuation Phase. Sputum results in the treatment card were supplemented from laboratory register, if required, and analyzed. Predictive values, sensitivity and specificity of smear were compared with culture results.

Results: The Negative Predictive Value (NPV) of smear was high from the 3rd month onwards (above 91%), at four months 98% or more and approached 100% from eight months onwards. The specificity of smear test gradually increased during treatment and from five months onwards, it was above 90%.

Conclusions: Considerable correlation was observed between sputum smear and culture during follow up of DOTS Plus treatment in the Intensive Phase. Accordingly, sputum smears can be recommended instead of culture.

[Indian J Tuberc 2010; 57:134-140]

***Key words*:** Sputum smear, Culture, MDR-TB, DOTS Plus.

INTRODUCTION

The emergence of strains of *Mycobacterium tuberculosis* resistant to antimicrobial agents is a worldwide problem. Multi Drug Resistant Tuberculosis (MDR-TB), defined as resistance to at least Isoniazid and Rifampicin, two of the most potent anti TB drugs, is a reflection of poor management of TB cases. A series of representative drug resistance surveillance studies from India have shown ~3% MDR-TB among new cases and 12–17% among cases with a previous history of anti-TB treatment. Several such studies are being undertaken in selected states in accordance with the WHO / IUATLD global surveillance of drug resistance project presently in the country [1].

The country has developed the National DOTS Plus guidelines for managing MDR-TB patients under the programme[2]. As per these, follow-up culture is done every month in the Intensive Phase (IP) and every quarter in Continuation Phase (CP). Each visit entails time, travel and work loss costs for the patient and the health system and also in many instances, prolongation of IP.

The most important objective evidence of improvement is the conversion of sputum smear and culture from positive to negative. Though smear conversion can be taken as an indicator, culture conversion which reflects viability of tubercle bacilli is more sensitive and is considered necessary to monitor progress in MDR-TB patients[2].

1. Assistant Medical Superintendent and Head, Department of TB Control & Training 2. HOD - TB & Respiratory Diseases 3.HOD - Department of Microbiology 4. Chest Physician 5. HOD - Department of Public Health 6. Chief Medical Officer 7.Research Officer 8. Director, LRSI 9. Statistician

LRS Institute of TB & Respiratory Diseases, New Delhi.

Correspondence: Dr. Rohit Sarin, Assistant Medical Superintendent and Head, Department of TB Control and Training, LRS Institute of TB & Respiratory Diseases, Sri Aurobindo Marg, New Delhi-110 030; E-mail: drsarin@yahoo.com; Phone: 0091-11-26865096, 0091-9999-71557; Fax: 0091-11-26517827, 0091-11-26517834, 0091-11-26568227

The National Guidelines rely primarily on culture reports for treatment regimen optimization i.e. shift from IP to CP and when to document cure, failure and stoppage of treatment. Culture result by the conventional method is not available before a lag period of approximately 6-8 weeks; therefore there is a delay in initiating any form of therapeutic action by the treating physician both for diagnosis and during treatment. In an effort to reduce this time, specially in resource poor settings, different diagnostic modalities are being researched such as Microscopic Observation Drug Susceptibility Assay (MODS)[3] and slide DST [4]. However, in the present study, an effort has been made to ascertain if simple sputum smear microscopy by Ziehl Neelsen method can be used for this purpose.

The LRS Institute is implementing a GLC approved DOTS Plus site in 1.8 million population of South Delhi, India[5]. Patients remaining smear positive at four months of Cat.II DOTS treatment were subjected to sputum culture and sensitivity and those with proven MDR-TB on DST were put on Standardized Treatment with Kanamycin, Cycloserine, Ethionamide, Pyrazinamide and Ofloxacin for 6-9 months of Intensive Phase (IP) followed by 18 months of Continuation Phase (CP) without Kanamycin and Pyrazinamide. The protocol for sputum examination is monthly during IP and every two months during CP. Shift from IP to CP was done only on three consecutive Sputum Culture negative reports during IP. Sputum Culture negative report only came after eight weeks. Hence, even if the time to Sputum Culture conversion was three months, these patients could be shifted to CP only when report of the 5th culture was received i.e. after seven months of IP. This needlessly prolonged treatment and also increased cost. Results of patients diagnosed with culture-proven pulmonary TB were retrospectively examined to ascertain if sputum smear could be used as a surrogate for culture thereby reducing the need for frequent cultures and hence save time and cost in the management of MDR-TB patients.

Smear microscopy is a rapid diagnostic tool which may allow the clinicians to take prompt and necessary decisions at site. It is important to determine whether the smear microscopy result is comparable to culture so that repeated cultures and concomitant delay can be avoided. The objective of this study was to assess the value of sputum smear and whether the same can be used as a surrogate marker for culture especially in the context of resource limited countries and in the absence of easy access to accredited culture laboratories.

MATERIAL AND METHODS

The study was carried out at the LRS Institute of TB and Respiratory Diseases in Delhi, India. This Institute has a National Reference Microbiology Laboratory and is implementing the DOTS strategy in a population of around 1.8 million. The Institute started its DOTS Plus project in 2002 in the same area, which was subsequently approved by GLC. The project had approval of Ethics Committee of the Institute. The initial results on the larger study in terms of treatment outcomes, defaults etc. have been published by Arora V.K. *et al*[5].

In the present study, the MDR-TB patients on DOTS Plus treatment and residing in the LRS Institute defined geographical area were included.

Table 1: Age and sex distribution of the evaluated patients

Age	Male	Female
0-14	2	6
15-24	12	32
25-34	30	17
35-45	18	4
45-54	10	1
55-64	4	-
>65	2	-
Total	**78**	**60**
GRAND TOTAL = 138		

Table 2: Concordance of Monthly Smear and Culture during Intensive Phase of DOTS Plus Treatment.

	(a)	(b)	(c)	(d)	
	S C	S C	S C	S C	
	+ + P P	+ - P N	- + N P	- - N N	**Total**
Month					
0 month	115	4	7	0	**126**
1st month	32	8	1	8	**49**
2nd month	35	14	6	31	**86**
3rd month	25	10	5	51	**91**
4th month	8	12	1	66	**87**
5th month	3	8	2	73	**86**
6th month	1	3	1	57	**62**
7th month	3	4	1	69	**77**
8th month	3	3	0	32	**38**
9th month	2	0	0	14	**16**

The data from January 2002 to March 2008 were scrutinized retrospectively. A total of one hundred and thirty eight case profiles of the MDR-TB patients on DOTS Plus treatment were screened during follow-up. The results of smear and culture as recorded in the treatment card in the intensive phase of treatment were analyzed. Wherever, there was a missing entry, effort was made to retrieve data from the Laboratory Register. Evaluable patients who had results of both smear microscopy and culture performed on the same sample on any occasion were only included. As this number varied in different months hence, the number of evaluable results also varied from month to month. There were 718 evaluable responses during the IP (6-9 months).

Smear microscopy was performed by the Ziehl- Neelsen method[6] and results were reported as per the National Guidelines[7]. Culture for mycobacteria was performed by the modified Petroff's method. The results of the smear microscopy and the culture were analyzed for each month of IP to see at what time of treatment the concordance of smear and culture could be considered acceptable[6,7]. The Positive Predictive Value (Proportion of Smear and Culture positive out of total smear positive) and Negative Predictive Value (NPP)(Proportion of Smear and Culture Negative out of the total Smear Negative) were ascertained. The sensitivity (proportion of smear and culture positive to total culture positive) and the specificity (proportion of smear and culture negative to total culture negative) were also ascertained.

RESULTS

The age and sex distribution of the 138 patients studied is as per Table-1. All the patients had received a Category-II Treatment Regimen

recommended by the National TB Programme and were sputum positive at four months or more of treatment (2 SHRZE)$_3$ (2 HRZE)$_3$ (5 HRE)$_3$. These patients were culture proven MDR-TB and had been put on the Standardized Treatment with 2nd line drugs as mentioned in the introduction. The culture conversion at 3rd and 6th month was 83% and 94% respectively. Overall treatment outcomes are available for 105 patients (cohort Jan. 2002 to March 2006) of which 61% were cured, 18% defaulted, 17% died, 3% failed and 1% was still on treatment.

Of the 138 patients, both smear microscopy findings and culture results were available on 718 instances during 6-9 months of IP and the same were evaluated. As shown in Table-2, only 227 out of 718 evaluated results were smear positive as well as culture positive for mycobacteria. With ongoing therapy, the patients gradually converted by smear and culture both, as is expected and at nine months only two results were smear as well as culture positive.

At start, the PPV of smear is high as most smear positive are culture positive, but with treatment, it decreases as chance of dead bacilli increases and smear positive can be culture negative. However, at nine months, when all/most dead bacilli are excreted, then smear positive will be culture positive so PPV will also increase. However, the figures being too small at nine months of therapy, the percentages of the same cannot be taken on face value.

After one month, the NPV of smear is high as smear negative reflects culture negative in most situations. It is observed that from the 3rd month onwards, it is above 91% and at four months it is 98% or more. The NPV approaches 100% from eight months onwards.

The specificity of smear test gradually increases during treatment and from five months onwards the specificity rises above 90%. This indicates that after the 5th month false positive with smear will be less. However, as the numbers are too small, the percentages cannot be taken on face value.

The sensitivity of smear is high at the beginning and near the end of IP indicating that the false negatives are low during this period. However, the sensitivity of smear is variable in between.

DISCUSSION

This 6-year retrospective review of data collected on patients with culture-proven pulmonary TB at LRSI of TB & RD enabled us to assess the value of examining multiple sputum specimens and the correlation between smear and culture during different months of treatment in order to determine at what step the patient could be shifted from the IP to the CP. With the present methodology of culture by LJ Media, a minimum of 8 weeks is required to report negative result. Hence, relying on culture results for making decisions of changing from IP to CP leads to unduly prolonging the IP thereby leading to delay in treatment completion and cost of treatment.

Analysis of records from the Damien project, Bangladesh, showed that on treatment about half of the positive smears do not correlate with a positive culture and may be due to dead AFB. In case of doubt, because of excellent clinical conditions, for instance, interpretation should rely on using additional smears after a few weeks to one month, which may be easier and faster than relying on culture[8].

AFB-microscopy is easy, rapid and highly specific. Moreover, the technique can easily be learned by paramedical staff, and high proficiency is possible with minimal training. Because the equipment is multi-purpose and widely available, this method permits accurate diagnosis to be made in laboratories worldwide.

Specificity of AFB-microscopy can reach over 99%, and may under ideal circumstances exceed that of culture. This is the case where evaluation centres on a diagnosis of AFB, not specifically MTB, and using re-checking as the gold standard. Follow-up smears are more difficult for finding AFB than diagnostic smears, for several reasons. If positive, most often they contain low

Table 3: Sensitivity, Specificity, PPV, NPV of Sputum Smear against Culture (as Gold Standard)

Month	Sensitivity (%age)	Specificity (%age)	PPV (%age)	NPV (%age)
0	94.3	--	96.6	--
1	97	50	80	88.9
2	85.4	68.9	71.4	83.8
3	83.3	83.6	71.4	91.1
4	88.9	84.6	40	98.5
5	60	90.1	60	97.3
6	50	95	25	98.3
7	75	94.5	75	98.6

numbers of AFB only. In addition, it is well known that AFB damaged by treatment is more difficult to stain with acid-fast staining. The recognition of positive follow-up smears thus requires a more perfect technique. The meaning of positive smears at follow-up examination is not always clear. This results from the inability of smear microscopy to distinguish living from dead AFB. Thus smear conversion lags behind culture conversion throughout treatment[8].

In the present study, the PPV is more in the initial few months as depicted in Table-3, but with appropriate therapy a steady decline was observed. Also, with the patient on therapy, the smear may be positive owing to the presence of dead bacilli. This fact is further supported by the evidence of culture being negative for that given sample. The variability in the PPV may be accounted for by the dead bacilli on the smear. The NPV shows an increasing trend starting from the end of the intensive phase 3rd month onwards. Thus indicating that at the end of three months of therapy if the patient is smear negative it can be predicted that the culture would likely be negative in 98-100% cases (Table-3); therefore, implying that if smear is negative after three months of IP, culture need not be done. This would obviate the need for repeated cultures, thereby reducing the burden on the laboratory and the expenditure incurred. Also, the clinicians need not wait till the culture reports are available i.e. a minimum of eight weeks before switching from I.P. to C.P.

The threshold for the smear to be positive is 10000 per ml of sample while for culture it is 100 bacilli per ml of sample[9-12]. Once the bacilli are exposed to antimicrobials, their cellular structure gets altered following damage; therefore there is very poor yield on culture indicated by either delayed growth or no growth at all.

In an earlier study by Lipsky *et al*,[13] the clinical value of microscopy for acid-fast bacilli (AFB) was assessed; the results of 3,207 clinical specimens submitted for mycobacterial smear and culture were analyzed. Mycobacteria grew from 176 (5.5%) of the specimens, 95 (54%) of which

were *Mycobacterium tuberculosis*. Although the overall sensitivity of the smear was low (33%), 65% of respiratory specimens yielding *M. tuberculosis* had positive AFB smears. Furthermore, 96% of patients with pulmonary tuberculosis from whom more than one specimen was processed had at least a single positive AFB smear. Smear sensitivity correlated well with quantitative growth; 89% of specimens yielding greater than or equal to 50 colonies per slant were smear positive. Further, after the results from culture-negative patients known to have active tuberculosis were eliminated from the analysis, the specificity of a positive smear rose to 98.3%. When the results of all specimens from each patient were considered *in toto*, the AFB smear had a predictive value of greater than or equal to 96% [13].

In the present study, the sensitivity was very high initially as the bacillary load was very high at the start of IP. After continuing the appropriate therapy, there is a variable trend probably due to dead bacilli detected on smear. The specificity is very high indicating that the frequency of false positives is very less.

In a landmark study conducted by Wallis *et al*, predictive models were developed to address the need for studying the outcomes of patients on therapy. Culture for mycobacteria, quantitative AFB smear, and CFU were very highly collinear (p ranging from 10^{-4} to 10^{-11}). The model proposed for low-income regions does not require mycobacterial culture but instead substitutes it with quantitative acid fast microscopy. The most serious potential limitation to these models here is the extent to which they can be implemented where the need is the greatest. The analysis indicates that the requirement for culture can be replaced by the use of quantitative acid-fast smear[14].

CONCLUSIONS

The AFB smear and culture results of all patients diagnosed with pulmonary TB were retrospectively analyzed in this study. There was a considerable degree of correlation between smear and culture during different months of treatment. As the NPV and specificity of smear were very high at four months onwards, the frequency of subjecting sputum samples for culture may be reduced.

We conclude that sputum smear could be used as a surrogate for culture thereby reducing the need for frequent cultures and hence save time and cost in the management of MDR-TB patients. However, cultures cannot be totally dispensed with and would be required from time to time to a limited extent as conclusive evidence of sputum conversion and cure. If smears are used in place of culture for defining change from I.P. to C.P., then this shift could be done much earlier because the conventional culture takes a long time. Accordingly cost of drugs, cost to health system and cost to patient can all be reduced.

The results of the study should be interpreted with precaution because of the very few number of subjects available for calculating the sensitivity and PPV. However, despite the small sample size, which was a limitation of this study, the implications may be far reaching. Thus, further long-term studies need to be carried out to validate the above discussed findings.

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Original article

ELECTROIMMUNOTRANSFERBLOT ASSAY FOR THE DETECTION OF MYCOBACTERIAL ANTIGENS IN THE CEREBROSPINAL FLUID FOR DIAGNOSIS OF TUBERCULAR MENINGITIS

K. Venkatesh and Subhash Chandra Parija

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Summary

Background: The diagnosis of tubercular meningitis (TBM) is often delayed as it presents initially with non-specific signs and symptoms leading to delay in appropriate therapy. Conventional modes of diagnosis are time-taking and immunodiagnosis has its own pitfalls. Antigen detection assays have been found to be quite promising in this aspect.

Aim: In the present study, attempts were made to evaluate the ElectroImmunoTransferBlot (EITB) test for detection of *Mycobacterium tuberculosis* antigens in CSF.

Methods: A total of 46 CSF specimens were collected from 26 clinically suspected cases of TBM and 20 non-TBM cases. The mycobacterial antigens were concentrated by immunoprecipitation and separated based on their molecular weight by SDS-PAGE which were further transferred and immobilized onto a matrix and detected by EITB.

Results: In TBM CSF specimens distinct bands of molecular weight 12kDa, 30-32kDa, 71kDa, 86kDa, 96kDa, 110kDa and 120kDa were seen in addition to 50kDa Immunoglobulin (Ig) heavy chain, 25kDa Ig light chain and an indistinct human albumin band at 69kDa. The control group CSF specimens also showed the Ig and albumin bands but showed no cross-reactive antigens. The following proteins 12kDa (7.7%), 30-32kDa (23%), 71kDa (19.2%), 86kDa (77%), 96kDa (57.5), 110kDa (23%) and 120kDa (15.4%) were identified as reactive bands. The results were compared to the reverse passive latex agglutination test.

Conclusion: The likelihood of diagnosing TBM as evidenced by detecting at least a single mycobacterium specific band was 88.4% by our protocol for antigen detection in CSF. The specificity of EITB for diagnosing TBM was found to be 100% when the 86kDa antigen was excluded from the analysis. However, the method of diagnosis is labour/reagent intensive and needs substantial validation. **[*Indian J Tuberc 2010; 57:141-147*]**

Key words: EITB, Tubercular meningitis, CSF, Antigen detection, *Mycobacterium tuberculosis*.

INTRODUCTION

Tubercular meningitis (TBM) caused by *Mycobacterium tuberculosis* is an important cause of mortality and morbidity in the developing world[1]. The diagnosis of TBM is often delayed as it presents initially with non-specific signs and symptoms. Although effective anti-tubercular agents are available for treating this condition, the treatment is often delayed[2,3]. On the other hand, patients with unrelated conditions are started on empirical anti-tubercular treatment which is unwarranted. Imaging studies are sensitive for diagnosing TBM pathology but are not specific. Demonstration of the acid fast bacilli in the cerebrospinal fluid (CSF) specimen is a rapid method for diagnosing the disease but is less sensitive[4]. Culture and isolation of *M.tuberculosis* from the CSF specimens though considered as gold standard takes time and the bacterial isolation rate is also poor.

Immunodiagnostic methods based on antibody detection can lead to erroneous false positive and false negative results. As the mortality and morbidity increase if the condition is not recognized and treated early, there is a need for a sensitive and specific, simple and rapid, antigen detection immunodiagnostic test for TBM. Many studies have used antibody-based assays [5-14]. Antigen

Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry.

Correspondence: Dr. Subhash Chandra Parija, Professor & Head, Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry-605006; Email: subhashparija@yahoo.co.in; Telephone: 91 413 2279214; Tele fax: 91 413 2279214

detection assays have been developed for many bacterial causes of meningitis including TBM. Most of the antigen detection assays are (Enzyme linked immunosorbent assay) ELISA based or (Radioimmunoassay) RIA based[15-21]. In the present study, attempts were made to evaluate the (Electro-immuno-transfer blot) EITB test for detection of *M.tuberculosis* antigens in CSF. We have also made an attempt to improvise the EITB test for antigen detection in CSF by concentrating the antigens by immunoprecipitation.

EITB is advantageous over ELISA based immunoassays as it is more specific, cross-reaching epitopes are less likely to be present in a protein with similar molecular weight which migrates to the same distance as the antigenic protein[22,23]. The sensitivity of EITB depends on the concentration of the specific proteins in the CSF, and the amount of other non-specific proteins present. If the concentration of antigens of interest is less, then they may not be visualized. On the other hand, when using other concentration techniques for concentrating the proteins, the non-specific unwanted proteins may also get concentrated and some of the antigenic activity of the specific proteins can be lost as they are denatured during this process.

MATERIAL AND METHODS

A total of 46 CSF specimens were collected from clinically suspected 26 TBM cases and 20 non-TBM cases[24]. This latter group included 20 patients and comprised seven culture proven bacterial meningitis, five cryptococcal meningitis, three cases of neonatal meningitis, two cases of neurocysticercosis and three with non-infectious CNS disorders. One to two ml of CSF was collected from the patients by doing a lumbar puncture under aseptic precautions. The CSF specimens were collected from both the clinically suspected cases of TBM and non-TBM cases. The CSF specimens were stored at -20^0 C till use. The mycobacterial antigen was prepared and the hyperimmune antimycobacterial antiserum was raised as mentioned previously[24,25]. The EITB was carried out as follows:

A. *Concentration of mycobacterial antigens by immunoprecipitation*

The myobacterial antigens in the CSF are concentrated by the method of immunoprecipitation. Immunoprecipitation was done according to the procedure described by Ed Harlow and Lane D [26] with modifications. To 100 µl of CSF 100 µl of antiserum was added. The mixture was incubated overnight at 4°C on a rotary shaker. The CSF antiserum mixture was centrifuged at 10,000 rpm x 5 min at 4°C and the supernatant containing soluble proteins were treated with protein A agarose. About 50 µl of protein A agarose (30%v/v in PBS-7.2) was added to the supernatant and incubated at 4°C for two hours to precipitate the antigen-antibody complex.

The precipitate was washed thrice with (phosphate buffered saline) PBS (7.2) by centrifuging at 10,000 rpm x 1 min. The supernatant was discarded and the pellet dissolved in 30 µl of chamber buffer and 10 µl of sample buffer and then kept in boiling water bath for five minutes. The resultant solution was centrifuged at 10,000 rpm x 10 min and the supernatant was used for (Sodium dodecylsulphate polyacrylamide gel electrophoresis) SDS-PAGE.

B. Separation of the antigens based on their molecular weight by SDS-PAGE

SDS-PAGE procedure was carried out as per the method described earlier[27]. Briefly, a 12.5% separating gel was cast by mixing 4.8ml of acrylamide stock to 3ml of separating gel buffer and 4.2ml of distilled water. To this mixture 50µl of 10% (Ammonium persulphate) APS was added and mixed thoroughly followed by 30µl of (Tetramethylethylenediamine)TEMED. The resultant solution was poured immediately between glass plates up to 2cm below the notch and the gel was allowed to polymerize for 30-60 min at room

temperature. The gel was overlaid with n-butanol to keep the gel surface flat. After polymerization butanol was removed and the surface was rinsed with distilled water. The stacking gel was prepared by mixing 1.34ml of acrylamide stock to 2ml of stacking gel buffer and 4.55ml of distilled water. To this mixture 40µl of 10% APS was added and mixed thoroughly followed by 20µl of TEMED. The resultant solution was poured over the separating gel and allowed to polymerize for 15-30 minutes. The comb was then removed and the wells were rinsed with distilled water.

The gel assembly was placed into the electrophoresis chamber with the notched plates facing inside and the upper and lower tanks were filled with running buffer. Then 20µl of the protein sample was loaded into each well along with molecular weight marker in one of the wells. Electrodes were connected to power pack and electrophoresis was done at a constant current of 20mA till the dye front reaches the separating gel and then the current was increased to 25mA. The run was stopped when the dye front reaches the bottom of the gel.The gel was carefully scooped from the glass plate and retained for blotting.

C. Transfer and immobilization of the separated proteins onto a matrix and detection of antigens by EITB

EITB procedure was carried out as per the method described elsewhere [28]. Briefly, the gel was marked to establish orientation and then soaked in transfer buffer. The nitrocellulose membrane was marked and soaked in transfer buffer for 15-20 min before transfer. Buffer chamber was rinsed with distilled water and frozen cooling unit and the Trans-blot electrodes were inserted. Then a 3 mm Whatman filter paper was placed in the transfer cassette on a fiber pad well soaked in transfer buffer. The gel was placed on the filter paper and then covered with a second set of filter paper and pad. The tank was then filled with transfer buffer followed

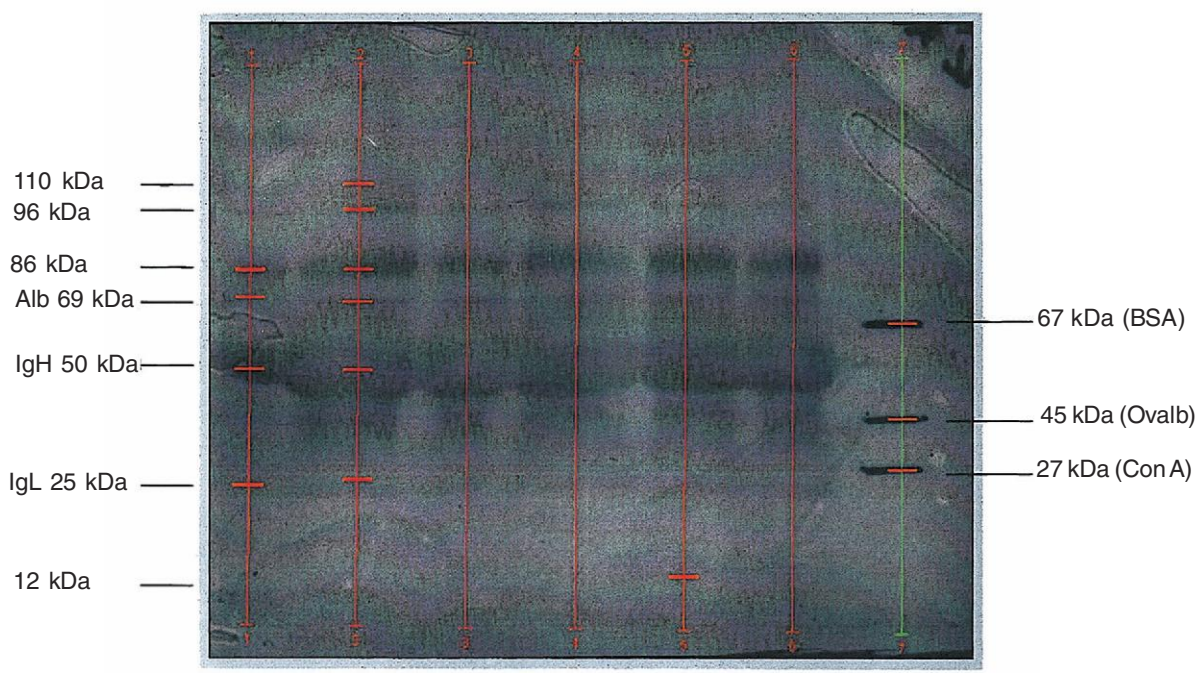

Figure: EITB of TBM cases showing reactive antigenic bands

Table: Evaluation of EITB for the detection of mycobacterial antigens in the diagnosis of TBM

Subject groups	No. of subjects	No.(%) of samples positive for Mycobacterial antigens by EITB						
		12 KDa	30-32 KDa	71 KDa	86 KDa	96 KDa	110 KDa	120 KDa
Clinically suspected cases of TBM	26	2 (7.7%)	6 (23%)	5 (19.2)	20 (77%)	15 (57.7%	6 (23%)	4 (15.4%)
Non TBM control cases	20	0	0	0	5 (25%)	0	0	0
Sensitivity	88.4%							
Specificity	100%							
Positive predictive value	100%							
Negative predictive value	100%							

by electrotransfer at 100V for one hour. The nitrocellulose membrane was then removed and treated with blocking buffer on a rocker shaker. The membrane was rinsed with wash buffer thrice and then probed with anti-mycobacterial antibody in (Phosphate buffered saline–Tween Bovine serum albumin)PBS-T BSA (1:100 dilution) overnight at 4°C. After washing thrice the membrane was treated with anti-rabbit IgG peroxidase (1:500, Genei, India) in 0.5% BSA PBS-T. The membrane was washed again and treated with the substrate solution. Colour development was stopped by washing the membrane in distilled water after 30min.The bands were captured by gel documentation system and their molecular weights were calculated by comparing with the standard markers.

Statistical analysis of the immunoassays

The sensitivity, specificity, positive predictive value and negative predictive value of the tests were calculated according to the method described by Galen and Gambino [29].

RESULTS

Electroimmunotransfer blot assay (EITB)

In a reactive EITB, the mycobacterial proteins in CSF specimen after immunoprecipitation with antimycobacterial antisera, separation and probing gave distinct bands. In the present study, the bands of molecular weight 12kDa, 30-32kDa, 71kDa, 86kDa, 96kDa, 110kDa and 120kDa were seen in TBM cases in addition to 50kDa Ig heavy chain, 25kDa Ig light chain and an indistinct human albumin band at 69kDa (Figure). The control group CSF specimens also showed the Ig and albumin bands. The control CSF specimens were also subjected to EITB, to rule out non-specific cross-reactive antigens.

The 86kDa antigen and the 96kDa antigens were the more frequent reactive bands present in 20 and 15 TBM patients respectively. Control CSF specimens from three cases of pneumococcal meningitis and two cases of cryptococcal meningitis showed reactivity at 86kDa. Hence this antigen is found to be non-specific for the diagnosis of TBM and was not included in calculating the efficacy of the test.

Table shows the sensitivity, specificity, positive predictive value and negative predictive value of EITB for diagnosis of tuberculosis meningitis.

DISCUSSION

Electro-immuno transfer blot (EITB) is an immunological technique which is used to detect

proteins immobilized on a matrix. The technique has been successfully applied to detect antigens and antibodies in body fluids in many infectious diseases. It has been employed to detect the antibody response against *M.tuberculosis* in CSF, in cases of tuberculus meningitis (TBM).

There is only one report, by Katti[30] with regard to antigen detection in un-inactivated CSF using EITB. Another study conducted by Mathai *et al*[28] made use of heat inactivated CSF, for antigen detection in CSF by EITB. One of the major limitations of antigen detection in CSF is the concentration of the antigen in CSF and the amount of CSF available for diagnostic purpose. A study on mycobacterial antigen detection using EITB in TBM cases, showed a varied sensitivity for different antigenic bands. The proteins of molecular weights 6 KDa, 12 KDa, 30-32 KDa,71 KDa, 82 KDa, 86 KDa, 96 KDa, 110 KDa and 120 KDa showed a sensitivity percentage of 26, 6, 98, 60,18,10,22 and 32 respectively [28,30]. In the study by Katti using un-inactivated CSF, one patient with a non-infectious neurological condition showed the presence of 86 kDa and 110 kDa antigens, while other non-neurological conditions and infectious neurological conditions were negative for antigenic bands in CSF[30]. The study by Mathai *et al*[28], using heat inactivated CSF, claims that 82 kDa mycobacterial antigen is sensitive and specific for diagnosing TBM.

Since the amount of mycobacterial antigens in CSF is very low for detection by EITB, and the quantity of CSF collected is also very less unlike other specimens, an attempt has been made in the present study to improvise EITB technique by immunoprecipitation of antigens in CSF followed by their separation and identification. In the present study, high molecular weight antigens were predominantly reactive. The proteins, viz. 12kDa (7.7%), 30-32kDa (23%), 71kDa (19.2%), 86kDa (77%), 96kDa (57.5), 110kDa (23%) and 120kDa (15.4%) were identified as reactive bands. The frequency of detection of the same antigens in the study performed by Katti is as follows: 12kDa (6%), 30-32kDa (98%), 71kDa(60%), 86kDa (18%), 96kDa (10%), 110kDa (22%) and 120kDa (32%). When comparing results of the present study with the EITB study reported by Katti[30] it is observed that more reactivity of the positive CSF specimens were found in the region of 86kDa and 96kDa proteins. These two antigens may be soluble proteins that get concentrated on immunoprecipitation hence the frequency of detection is increased. But the 86kDa protein in the present study was found to be non-specific.

In the present study, frequency of detection of 30-32kDa and 71kDa antigens were less compared to that reported by Katti[30]. The latter study has shown that these two antigens are cell wall associated antigens and not seen in secreted culture filtrate proteins. The reason for lower sensitivity of detection by EITB in our present study could be that these cell wall associated antigens get sedimented during the centrifugation process involved in the method to get a supernatant free of cell debris, which was then treated with the antiserum. Another possible reason could be that protein A agarose binds only to IgG antibodies, hence antigens having more affinity and avidity to IgM antibodies may not be detected by this method. These may be the reasons for failure to detect low molecular weight 6kDa antigen in the present study.

The likelihood of diagnosing TBM as evidenced by detecting at least a single mycobacterium specific band was 88.4% by our protocol for antigen detection in CSF. The specificity of EITB for diagnosing TBM was found to be 100%, when the 86kDa antigen was excluded from the analysis. Three patients who were started on ATT before the CSF specimens were collected were reactive only to the 96kDa antigen. All the three cases were negative by RPHA. One case where no antigenic bands were detected improved with treatment. The four cases that could not be detected by EITB were also negative by RPHA. **These findings suggest that the levels of antigens in CSF decrease with treatment and antigen detection can be used as a prognostic marker also. There is a significant difference in the outcome of the disease if diagnosed and treated in stage II. Hence early antigen detection and early treatment possibly would reduce morbidity and mortality due to TBM to a great extent.**

The EITB technique improvised by using immunoprecipitation technique was found to be sensitive and specific for diagnosing TBM. EITB results are available in two to three days hence it can be considered as a rapid diagnostic assay for TBM. Although EITB is not a simple technique it can be used to evaluate and standardize other simple techniques for diagnosis TBM. The specific antigens can be characterized and made use of, in ELISA based assays. The prognostic value of EITB needs further study in a larger patient population, over a long period of time, to assess its true efficacy.

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Case Report

MYCOBACTERIUM AVIUM BACTEREMIA AND DUAL INFECTION WITH *MYCOBACTERIUM AVIUM* AND *MYCOBACTERIUM WOLINSKYI* IN THE GUT OF AN AIDS PATIENT – FIRST CASE REPORT

Rahul Narang, Pratibha Narang, A.P. Jain, D.K. Mendiratta, Rajneesh Joshi, Ram Das and V. M. Katoch

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Summary: An AIDS patient was admitted to a tertiary care hospital in central India with fever, weight loss, breathlessness, night sweats, diarrhoea, BMI 14kg/m^2, Hemoglobin 8gm% and CD4 counts 120 cells/cumm. His blood culture by BACTEC 460 TB system revealed *Mycobacterium avium* bacteremia and stool culture grew *Mycobacterium avium* and *mycobacterium wolinskyi*. ***[Indian J Tuberc 2010; 57:148-151]***

Key words: *Mycobacterium avium bacteremia, Mycobacterium wolinskyi, Dual infection, Non-tuberculous mycobacteria*

INTRODUCTION

Non-tuberculous mycobacteria (NTM), also known as atypical mycobacteria or mycobacteria other than *Mycobacterium tuberculosis* (MOTT) have been recognized since Koch's time but being opportunists did not gain much importance for long time. However, today the recovery of NTM from patient's specimens from sites where they can cause infections called "other mycobacteriosis", is of concern to microbiologists and physicians alike and the focus has been gradually shifted from AFB with rough, tough and buff colonies of *M. tuberculosis* to AFB with smooth and pigmented colonies and some with rapidly growing colonies. NTM infections are more common in developed countries but have also been documented in developing countries of Latin America, Africa, and Asia[1-5]. Many a time the NTM are found circulating in blood (mycobacteremia) and lead to disseminated infections. This situation is more closely related with immunosupressed states and particularly with AIDS. Among disseminated NTM infections, most are caused by mycobacteria belonging to *Mycobacterium avium* complex (MAC) and are known as Disseminated MAC (DMAC). In India, very few studies have been undertaken on NTM and the data is scarce. The data on disseminated disease in HIV/AIDS patients is even scarcer. Till date there are only three published studies that have proved disseminated NTM disease in AIDS patients[5-7].

Mycobacterium wolinskyi is a rapidly growing mycobacterium that belongs to the *M. smegmatis* group, which includes *M. smegmatis* sensu stricto and other two species, *M. goodii* and *M. wolinskyi* described in 1999. Only a few cases of infection caused by *M. wolinskyi* have been reported[8-10], and these included three cases of bone infection and one case of infection of a hip prosthesis. All patients had a history of surgery after traumatic injury and all specimens were isolated from the surgical wound. In only one study, was there a report of bacteremia caused by *M. wolinskyi*[11]. There is no report of its isolation from the gastrointestinal tract (GIT).

We present here a case report of an AIDS patient with dual infection of the gut with *M. avium* and *M. wolinskyi* along with isolation of *M. avium* from the blood.

CASE REPORT

A 30-year-old male, HIV seropositive, person was admitted to a tertiary care rural Kasturba Hospital, Sevagram situated in central India with history of fever of three months' duration associated with loss of weight, breathlessness, night sweats

Kasturba Hospital, Mahatma Gandhi Institute of Medical Sciences (MGIMS), Sewagram, District: Wardha (Maharashtra)
Correspondence: Dr. Rahul Narang, Professor, Department of Microbiology, MGIMS, Sewagram-442102, Dist. Wardha, (Maharashtra)

and diarrhoea. There was no history of cough. He was asymptomatic at the time of his initial HIV diagnosis one year ago, and no further evaluations were done at that time and neither any anti-retroviral treatments were initiated. At the time of admission, patient was emaciated (BMI 14kg/m^2), and initial clinical examination had no evidence of opportunistic infections (absence of oral thrush, lymphadenopathy, any skin lesions, or enlargement of liver or spleen). His chest radiography and abdominal ultrasound did not show any positive finding. He had a low CD4+ count (120 cells/ µl; CD8/CD4 ratio 12.68), and had normocytic anaemia (Hb 8gm%, MCV 80fl).

As part of an ongoing project approved by local Ethics Committee, the blood of the patient was cultured for mycobacteria using BACTEC 13A medium, after obtaining written consent. Vials were read using BACTEC 460TB system and subcultures were made on BACTEC 12B medium, Lowenstein Jensen medium (LJ) and paraffin slide culture system (PSC) as per previously reported methodology[5]. In PSC, a paraffin coated slide dipped in sucrose free Czapek broth with BACTEC PANTA, acts as a sole source of carbon and energy and permits the growth of NTM on the paraffin slide from the inoculated surrounding fluid. It does not support the growth of *M. tuberculosis*. Two stool samples of the patient were collected and were processed as per the methodology adopted in another study using LJ and PSC[12]. Sputum was not examined as the patients did not have signs and symptoms of chest involvement.

The blood sample of the patient showed growth of *M. avium*. In addition, *M. avium* along with *M. wolinskyi* was isolated from two of his stool samples collected on two consecutive days. The isolates were confirmed by sequencing of a part of 16S rRNA gene region nt-28-341 of *E. coli*[13]. The *M. avium* isolates from blood and stool were also typed using PCR for IS 1245 and IS 1311 and were found to be the same strain. The drug resistance pattern of both the *M. avium* strains was also the same. They were only sensitive to azithromycin and showed resistance to streptomycin, isoniazid, rifampicin, ethambutol, ofloxacin and kanamycin. However, the patient was lost to follow up by the time the diagnosis of disseminated MAC could be established.

DISCUSSION

Infection with multiple NTM species[14, 15] or multiple strains of the same species[16, 17] in AIDS patients have been reported from the West. However, to the best of our knowledge, this is the first case report on dual infection of the gut with *M. avium* and *M. wolinskyi* and *M. wolinskyi* is being reported from India for the first time.

Infections with *Mycobacterium avium* Complex (MAC) have been prominent particularly after the increase of such infections in AIDS patients reported from the West[14, 17]. Even in the pre-AIDS era, these were major cause of pulmonary and other infections[18]. Few studies from India have reported MAC in AIDS patients. From our hospital, two previous studies have reported isolation of *M. tuberculosis*, *MAC*, *M. fortuitum* and *M. simiae* in various clinical samples (blood, sputum and stool) of AIDS patients[5,12]. Recently, Singh *et al* have reported five isolates of *M. avium* in Indian AIDS patients[19]. A single case with MAC infection was detected among 23 HIV infected cases with pulmonary TB and pleural effusion in a study conducted in Kolkata[20]. Opportunistic infections by MAC in HIV infected patients, though common in adults, are rarely seen in infants. An HIV seropositive infant who presented with isolated axillary lymphadenitis was diagnosed to be lymphadenitis due to MAC in Berhampur[21]. In a case report published from New Delhi, a 27-year-old HIV-seropositive man with diarrhoea had *M. avium-intracellulare* isolated from his stool sample[22]. Our patient with NTM in his gut did not suffer from diarrhoea.

Studies conducted by some researchers using non-radiometric culture techniques and pulsed field gel electrophoresis (PFGE) have shown that as many as 24% of AIDS patients with disseminated MAC infection have polyclonal infection, that is, are infected simultaneously with two different strains of *M. avium*[16, 17]. Further, *M. avium* may cause

mixed infections along with other NTM such as *M. kansasii* and *M. simiae* etc[14, 15]. We have not come across any report of isolation of *M. avium* with *M. wolinskyi*. This could be the first report about simultaneous isolation of *M. avium* and *M. wolinskyi* from AIDS patient in the world.

M. wolinskyi is a rapidly growing mycobacterium belonging to *M. smegmatis* group. *M. wolinskyi* and *M. goodii* are the novel species belonging to this group and are most often associated with post-traumatic or post-surgical wound infections including osteomyelitis[23]. These are susceptible to sulfamethoxazole, amikacin, imipenem and the tetracyclines, variably resistant to clarithromycin, and intermediately resistant (*M. goodii*) or resistant (*M. wolinskyi*) to tobramycin. The three groups, viz. *M. smegmatis*, *M. goodii* and *M. wolinskyi* are similar by routine biochemical and growth characteristics, but have different mycolic acid dimethoxy-4-coumarinylmethyl ester elution patterns by HPLC and different PCR-restriction enzyme patterns of a 439 bp fragment of the *hsp-65* gene. *M. wolinskyi* isolates differ from *M. smegmatis* by 18 bp by 16S rRNA sequencing and exhibit 25% homology by DNA–DNA hybridization[8]. In the present study *M. wolinskyi* was also identified by 16S rRNA sequencing.

Generally, NTM colonise the gut or act as passengers. In this case both the NTM were isolated twice but only in two consecutive days' stool samples. Thus it is difficult to attribute pathogenic status to *M. wolinskyi* in this case, particularly when the patient had no gastrointestinal symptoms and when *M. avium* was isolated from blood and *M. wolinskyi* was not. There is one report so far to suggest that *M. wolinskyi* can cause mycobacteraemia[11]. The fact that *M. wolinskyi* is a rare isolate and with not much data on the organism and that both *M. avium* and *M. wolinskyi* have clinical significance shows that the information on dual isolation of the two from the gut and *M. avium* from the blood is relevant. It also reports the presence of *M. wolinskyi* in India. The patient was lost to follow up due to delay in the diagnosis and therefore proper treatment for *M. avium* could not be instituted.

In summary, even in India, *M. avium* bacteremia should be suspected in AIDS patients with fever and efforts should be made for isolation and rapid identification of the organisms from as many sites as possible.

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Case Repot

LIVER TUBERCULOSIS IN AN HIV PATIENT : DIAGNOSIS AND MANAGEMENT

D. Bendayan , K. Litman, A. Hendler and V. Polansky

(Received on 23.12.2009; Accepted after revision on 1.6.2010)

Summary*:** Hepatic involvement is common in miliary and extra-pulmonary tuberculosis but is usually clinically silent. Therefore, it is rarely diagnosed. We report the case of a patient that presented with prolonged fever and hepatomegaly. Liver biopsy revealed non-necrotizing granulomas that led in turn to the diagnosis of generalized tuberculosis and HIV infection. The patient reported an old untreated tuberculosis and depression of the immune system provoked the reactivation of this old tuberculosis focus. We describe the clinical course of the disease and the challenges associated with the complexity of the treatment. Diagnosis of hepatic tuberculosis requires a high degree of suspicion especially in AIDS patients who show atypical presentations. However, it is a potential curable disease and good results have been obtained with the four drug regimen. ***[Indian J Tuberc 2010; 57: 152-156]

Key words: Liver granuloma, HIV, Tuberculosis, FUO

CASE PRESENTATION

A 33-year-old woman was hospitalized for investigation of prolonged fever, abdominal pain, weakness and non-productive cough.

She reported a history of an old pleural effusion that was only drained without any antibiotic treatment and was an intra venous drug addicted, but stopped it many years ago. She denied HIV infection by a negative serological test six years ago.

The physical examination was unremarkable except for an enlarged, firm and tender liver (7cm below the right costal margin). Laboratory results showed a high serum level of alkaline phosphatase (more than ten times the normal), normal serum bilirubin level, positive HCV antibody, mild anemia and elevated sedimentation rate (Table). The chest radiograph showed pleural thickness without parenchymal infiltrate. The microscopic examination of the sputum was negative for Acid fast bacilli (AFB). Diffuse hepatic infiltration was noted on the abdominal CT and the liver biopsy revealed non-caseating granuloma formation with large epithelioid cells that did not stain for AFB and PAS (Figure). Our serological test for HIV was positive (ELISA and Western Blot tests) with CD4+ T cells number of 200 cells/mm³ and Viral Load of 210.000 IU. In a patient with HIV infection, the main cause of liver granuloma is mycobacterium infection, so we started on anti-tuberculosis treatment (Isoniazid, Rifampicin, Pyrazinamid, Ethambutol) that provoked an immediate and spectacular decrease of fever. Progressively, the abdominal pain diminished and the liver size became smaller. Two weeks later, sputum and urine cultures revealed *Mycobacterium tuberculosis* organisms sensitive to the first line drugs. Gradually, the liver function returned to normal and the patient felt better. Two months after anti- tuberculosis treatment, another CD4 T cell test revealed a decrease of the number to 35 cells/mm³. We started anti retroviral therapy with a regimen that included Efavirenz, Emtricitabine and Tenofovir. Two weeks after this combination, the patient complained of fever, weakness and a new infiltrate was found in the left lower lobe of the lung. The bronchoalveolar lavage fluid (BAL) examination revealed numerous acid fast specimens and later, *Mycobacterium tuberculosis* was recovered on culture. Following the finding of new AFB in the BAL, we added second line drugs (Amikacin and Levofloxacin) to exclude treatment failure. However,

Pulmonary and Tuberculosis Department, Shmuel Harofe Hospital, Beer Yaakov, affiliated to Sackler Faculty of Medicine
Correspondence: Dr Bendayan Daniele, Pulmonary and Tuberculosis Department, Shmuel Harofe Hospital, Beer Yaakov 70300 Israel. Tel: 972-8-9258693; Fax: 972-8-9258700; E-mail: daniellbd@hotmail.com

Table: Laboratory results

ESR (ml /H)	140	Natrium (meq /L)	131 (135-145)	Alkaline Phosphatase (u/l)	1980 (100-290)
Hemoglobin (g/dl)	9	Potassium (meq /L)	3.8 (3.3-5.1)	Gamma GT (u/l)	1138 (7-32)
WBC ($10^3/\mu l$)	7200	Urea (mg/dl)	29 (10-45)	AST (u/l)	42 (11-39)
Neutrophils (%)	81	Creatinine (mg/dl)	0.6 (0.7-1.2)	ALT (u/l)	78 (9-37)
Lymphocytes (%)	7	Protein (gr/dl)	6.9 (6-8)	Total Bilirubin (mg/dl)	1 (0-1.3)
Platelet count ($10^3/\mu l$)	291.000	Albumin (gr/dl)	2.9 (3.5-5.5)	Coagulation test	Normal

ESR: Erythrocyte Sedimentation Rate
WBC: White Blood Cells count
Gamma GT: Gamma Glutamyl Transpeptidase
AST: Serum Aspartate Aminotransferase
ALT: Serum Alanine Aminotransferase (u/l)

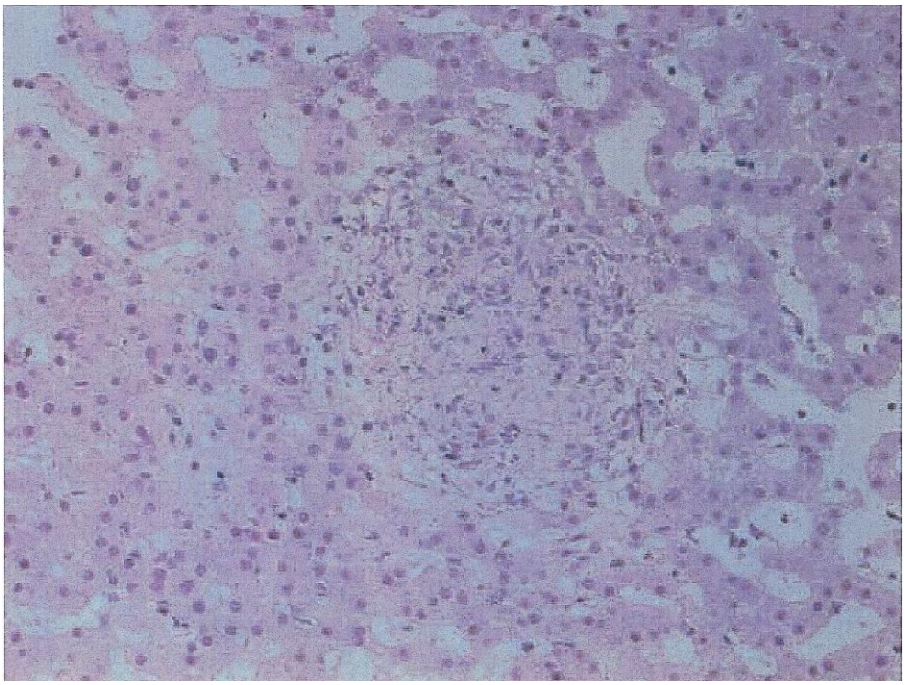

Fig. Liver biopsy revealing non-caseating granuloma formation

it had no effect on the general condition of the patient and fever, and finally, we attributed the symptoms to the Immune Reconstitution Syndrome and corticosteroid (Prednisolone 40 mg) was added successfully. The patient went on with the treatment without any other side effects. Corticosteroid was decreased progressively after one month and the sputum culture became negative for MTB. She went on with this combined regimen for a total of nine months.

DISCUSSION

Hepatic granuloma is a frequent finding of liver biopsies (incidence of 2 to 15%), and is caused by a variety of etiologies depending on the patient's characteristics and geographical location[1]. While immunological diseases like Primary Biliary Cirrhosis (PBC) and sarcoidosis are frequent causes of hepatic granulomas in immunocompetent patients and in the Western hemisphere, infectious agents, especially Mycobacterium specimens (*MAC*: *Mycobacterium Avium Intracellulare* and *MTB*: *Mycobacterium tuberculosis*) are the main causes of hepatic granulomas in patients from Asia and Africa and in HIV infected patients[2]. Tuberculosis was responsible for 55% and 32% of hepatic granulomas in India and in Saudi Arabia respectively, but other infectious diseases (Brucellosis, Leishmaniosis, Hydatidosis) have also been reported to cause liver granulomas.

Tuberculosis involves the liver mainly through hematogenous spread and is rarely an isolated disease. Three types of hepatic tuberculosis have been described: diffuse hepatic involvement in the context of disseminated miliary tuberculosis, diffuse hepatic infiltration without any known pulmonary involvement and focal abscess of the liver. The most common form is the first one but lack of familiarity with the disease led to its discovery on autopsy or surgery[3-5].

Fever, weight loss, abdominal pain and disproportionate elevation of the serum alkaline phosphatase level are the most common presenting features. Radiological features are not specific and liver needle biopsy confirms the diagnosis by demonstration of epithelioid granuloma formations in 80-100% of cases[6].

Hepatic tuberculosis is a potentially curable disease and is treated like other extra-pulmonary tuberculosis with the use of four drugs during the initial two months followed by two drugs in the next seven months. The hepatotoxicity side effects of the drugs are a dilemma for treatment that should be managed by an expert team. New liver biopsy may help to clarify worsening hepatic parameters.

Active tuberculosis is an AIDS defining disease. The immune suppression induced by HIV modifies the clinical course and the presentation of the disease[7]. Therefore, extra-pulmonary tuberculosis is common (50% versus 15% in immunocompetent patients) and the liver is often involved besides lymph nodes and pleura as it was reported in many retrospective studies[3-5]. The addition of antiretroviral treatment (HAART) during the course of the antituberculosis treatment has been proved to reduce HIV progression, opportunistic infections and mortality, but treatment combination is complex and is associated with increased drug toxicity (especially liver toxicity), drug-drug interaction and the immune reconstitution syndrome (IRIS). Rifampicin has a key role in the anti-tuberculosis treatment but its interaction with the antiretroviral drugs (especially the Protease Inhibitor and Non Nucleoside Reverse Transcriptase Inhibitors) is problematic as it decreases therapeutic activity and leads to HIV resistance. The WHO recommends regimen including two Nucleoside Reverse Transcriptase Inhibitors (NRTI) and one Non Nucleoside Reverse Transcriptase Inhibitor (NNRI) that allow normal dose of Rifampicin without any significant interaction with the antiretroviral drug concentration[8, 9].

Our patient was prone to develop hepatotoxicity as she was coinfected by hepatitis C and received the combination of HAART and anti-tuberculosis drugs. The prevalence of HCV infection in HIV infected patients is high (15 to 35% in US and Europe with an excess of 80% in drug users). HCV impairs hepatocyte defense mechanism and is a risk factor for lung injury during anti-tuberculosis

treatment as well as following HAART institution[10,11] .Data obtained from prospective studies indicate a 2 to 10 fold chance of elevated liver enzymes (3.5 - 5 times the upper limit of the normal) in HCV positive patients in comparison to HCV negative patients. On the other hand, elevation of serum bilirubin level (> 3mg/dl) occurs only in 5% of treated patients[12]. In regard to the anti-tuberculosis treatment, Kwon *et al* reported the elevation of liver enzymes level until 120IU/L during standard therapy in 41% of seropositive HCV patients in comparison to 20% control and over 120 IU/L in 13% in comparison to 4% in HCV negative patients. They also showed that treatment reintroduction after resolution of the hepatotoxicity is feasible but requires monthly liver test function[13].

In our patient, the liver function improved soon after the beginning of the anti-tuberculosis treatment and there was no aggravation during HAART despite the HCV coinfection and drugs interaction.

Hepatic granuloma was the presenting symptom of generalized tuberculosis and HIV infection. The tuberculosis infection arose as the immune system depressed and reactivated in an extra pulmonary site. The antituberculosis treatment institution was unremarkable but the recrudescence of fever and the new lung infiltrate that appeared after HAART institution was attributed to IRIS. IRIS developed in about 30% of patients following HAART institution, especially if the CD4 cells are less than 200 cells/mm³. It results from the recovery of the immune system with reconstitution of the antigen specific T cell mediated immunity. As the CD4 cell count rapidly increases, non-specific symptoms such as fever, nodal enlargement, worsening or occurrence of new pulmonary infiltrates, appear. There are two types of presentation: unmasking of undiagnosed tuberculosis or deterioration of existing lesions. The diagnosis is of exclusion and treatment failure, drug resistance or other opportunistic infections need to be ruled out[14,15].

Following the appearance of a new lung infiltrate, drug resistance was suspected on the basis on new AFB in BAL, and we added second line antibiotic drugs without clinical improvement. A course of steroid led to improvement and the symptoms were attributed to IRIS.

Hepatic tuberculosis should be always suspected in the presence of hepatomegaly, fever of unknown origin and a disproportionate elevation of alkaline phosphate serum level, especially in HIV infected patients. Granuloma formation is the most sensitive diagnosis feature. Combination of HAART and anti-tuberculosis drugs reduces mortality and is the key of successful outcome, especially when managed with an expert team.

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Dr. Vijay Kumar Dhingra, Director, New Delhi Tuberculosis Centre, passed away on 19th May, 2010.

A doyen of chest and respiratory diseases, Dr. Dhingra had to his credit many awards and recognitions of the Tuberculosis Association of India (TAI), served on its various committees and contributed a lot to TAI in general and the New Delhi Tuberculosis Centre, in particular.

Dr. Dhingra was the Associate Editor of *Indian Journal of Tuberculosis* and took keen interest in its publication by way of contributing and reviewing articles of rich research value.

His demise 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.

Case Report

LUPUS VULGARIS OF EXTERNAL NOSE WITH SEPTAL PERFORATION- A RARITY IN ANTIBIOTIC ERA

Ajay Garg[1], Raman Wadhera[2], S.P. Gulati[3] and Jagjit Singh[4]

(Received on 11.12.2009; Accepted on 1.6.2010)

Summary: Lupus vulgaris (LV) is the commonest morphological variant of cutaneous tuberculosis. Case of LV of external nose extending to internal nose causing septal perforation is documented here. Histopathology of biopsy taken confirmed the diagnosis of LV. Patient responded well to Anti-tubercular therapy (ATT). ***[Indian J Tuberc; 2010: 57:157-159]***

Key words: Lupus vulgaris, Septal perforation, ATT.

INTRODUCTION

Tuberculosis (TB) poses a major health problem in India. Poor living conditions, overcrowding, poverty, malnutrition, illiteracy and resurgence of HIV infection are some factors which attribute to this burden. Tubercular infection varies from pulmonary to extrapulmonary TB. Among these various forms, cutaneous TB accounts for about 1.5% cases of extrapulmonary TB.[1] Cutaneous TB can have varied presentations. LV is the commonest morphological variant of cutaneous TB characterised by its chronic, indolent & tissue destructive nature. In modern antibiotic era, LV of external nose extending to internal nose causing septal perforation is a rarity.

CASE REPORT

A 10-year-old male presented with progressive & non-healing lesion over dorsum of nose for past three months. He was under treatment with oral antibiotics and topical applications, but without any improvement. According to parents, disease initially started with a small ulcerative lesion over the dorsum of nose & slowly progressed to involve whole of dorsum. Examination revealed pigmented macular lesion over whole nasal dorsum extending to both alae (Fig. 1). There was no cervical lymphadenopathy. Nasal endoscopy revealed crusting in both the vestibules and perforation in the cartilaginous septum. Computed tomography scan showed perforation in the cartilaginous septum (Fig. 2). His family history and personal history was

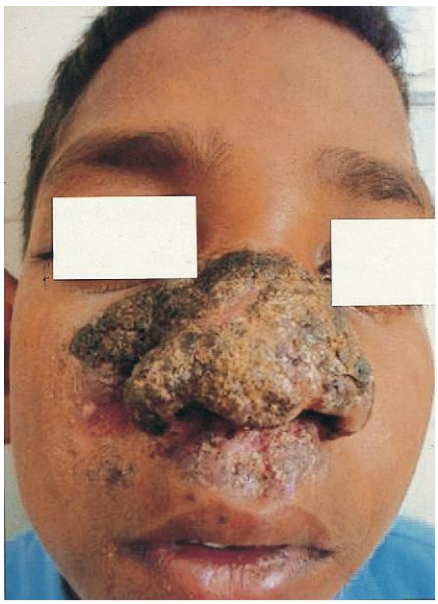

Fig. 1: Lupus Vulgaris lesion over whole dorsum of nose extending to both alae & right cheek.

1. Senior Resident 2. Professor 3. Senior Professor & Head 4 Assistant Professor
Department of Otorhinolaryngology & Pediatrics, PT.BDS PGIMS, Rohtak (Haryana)
Correspondence: Dr. Ajay Garg, 16, Bank Colony, Behind I.T.I, Rohtak-124001 (Haryana), Mobile- 093155-21969, 01262-230140; E-mail: dr_ajay_garg@yahoo.co.in

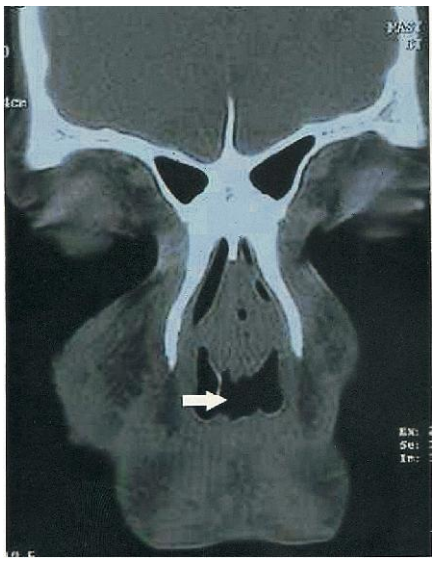

Fig. 2: CT scan of nose showing perforation in the cartilaginous septum (White arrow).

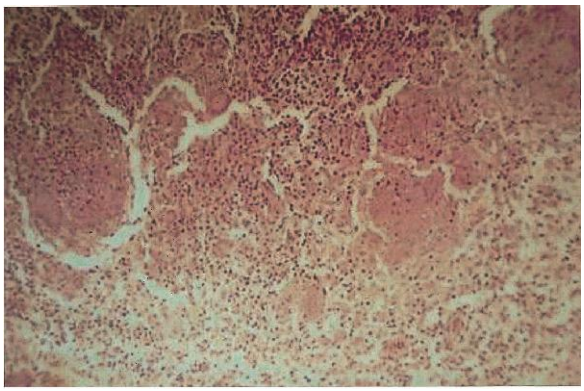

Fig. 3: Micrograph showing non-caseating tuberculoid granulomas and Langhan's giant cells (H&E X10).

unremarkable. Haematological investigations were inconclusive except for raised ESR which was 44 mm 1st hr. X-ray chest was also normal. Deep biopsy specimens were taken from (a) dorsum of nose and (b) margins of septal perforation and submitted for histopathology. Histopathological features were consistent with the diagnosis of LV showing non-caseating tuberculoid granulomas and Langhan's giant cells (Fig. 3). Ziehl-Neelsen stain (ZN stain) didn't reveal AFB bacilli in the specimen. Patient's PCR assay was negative.

Patient was treated by initial two months intensive phase with four drug regimen of isoniazid (4-6 mg/kg), rifampicin (8-12 mg/kg), ethambutol (15-20 mg/kg) and pyrazinamide (20-30 mg/kg) followed by four months of continuation therapy with two drugs, isoniazid and rifampicin (2 HRZE/ 4HR). Patient had significant relief in first three months of treatment, unfortunately patient lost to follow-up afterwards.

DISCUSSION

LV is a chronic, indolent and progressive form of cutaneous TB characterised by tissue destructive nature, found twice as commonly in females as in males & most often in early adulthood. It is a disease of northern climates and is rare in the tropics, reason being it needs a cold and moist climate.[2] In Indian subcontinent, the trunk is commonly involved site whereas in children lower extremities and gluteal region are commonly affected. In European countries, head and neck region is commonly involved.[3]

LV occurs either by haematogenous or lymphatic route from a distant focus or by direct inoculation of the bacilli. Cases at the site of BCG vaccination have also been reported.[4] LV lesions commonly occur on previously normal skin. The mucocutaneous junction of the nasal septum is the commonest site of inoculation of bacilli as this is frequently exposed to trauma in patients who have the habit of picking the nose. The disease may extend further inwards to involve mucous membrane of internal nostril, affecting mainly the anterior cartilaginous portion of the septum and anterior ends of the turbinates.[2] External lesions may also extend to involve mucosa of internal nose as happened in our case. Typical lesions of LV are characterised by a well demarcated, skin coloured, or erthyematous plaque with deep seated tiny nodules that can be seen as yellow-brown macules (apple jelly-coloured

nodules) on diascopy. Peripheral extension and central healing is hallmark of LV lesions. Various clinical variants of LV include (a) the classic plaque or keratotic type, (b) the hypertrophic type, (c) the ulcerative type, (d) the atrophic type, and (e) mutilating type. The plaque or keratotic type is most commonly found.[1]

Histopathology of biopsy specimen is diagnostic but sometimes biopsy specimen may reveal non-specific inflammatory changes indicating biopsy from superficial lesion thus missing the diagnosis, so a deep biopsy specimen must be taken to reach at a final diagnosis. Microscopy of tissue sections shows tuberculoid granulomas and Langhan's giant cells with slight or absent caseation. Fibrosis may be seen in the area of scarring and healing. Bacilli are scanty and difficult to demonstrate.[5] Diagnosis may be confirmed by Polymerase Chain Reaction (PCR) for *Mycobacterium tuberculosis*. LV must be differentiated from discoid LE, sarcoidosis, pseudolymphoma, tertiary syphilis, deep fungal infections, leprosy, lupoid leishmaniasis, and chronic pyodermas, and Wegener's granulomatosis.[6]

LV of external nose may cause dacryocystitis, corneal ulceration, nasopharyngeal lupus or lupus of the face. There may be extensive scarring and distortion of the nasal vestibule, tip and alae nasi. When internal nose is involved, it may sequel to atrophic rhinitis. Epithelioma may develop in the infected tissue.[2] LV lesions may also complicate to malignant conditions like squamous cell carcinoma.[7]

Lesions such as LV of head and neck area must be diagnosed as early as possible, especially in paediatric age group owing to the fact that progression of disease may lead to severe cosmetic morbidity. A deep biopsy must be subjected to histopathology to rule out chances of misdiagnosis, delayed diagnosis and in turn any cosmetic morbidity.

In India, TB awareness programmes are concentrating mainly on pulmonary cases. In general population, lack of awareness for extrapulmonary cases leads to their delayed presentation to the clinicians, thus increasing the chances of disease associated morbidity.

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Case Report

ERYTHEMA INDURATUM - A TYPE OF CUTANEOUS TUBERCULOSIS

C. Nirmala[1] and A.H. Nagarajappa[2]

(Received on 5.11.2009; Accepted on 16.2.2010)

Summary: We report a case of erythema induratum recently encountered in our centre. A 14-year male presented with history of fever, weight loss and multiple, painful, hyperpigmented patches over both legs and dorsum of foot, since six months. FNAC showed evidence of granulomatous inflammation. Biopsy of the lesion showed skin with inflammatory infiltrate in the deep dermis composed predominantly of epithelioid granulomas, Langhan's giant cells and mature lymphocytes. A strongly positive Mantoux test and elevated TB IgG and IgM antibody levels suggested tuberculosis . The patient responded well to a course of anti-tuberculous therapy with marked resolution of the lesions. ***[Indian J Tuberc 2010; 57:160-164]***

Key words: Erythema induratum, Cutaneous tuberculosis, Hypersensitivity reaction.

INTRODUCTION

Erythema induratum remains one of the rarely encountered tuberculid, although tuberculosis is known to be endemic in the developing countries.

In 1861, Bazin gave the name 'erythema induratum' to a nodular eruption that occurred on the lower legs of young women with tuberculosis. In 1945, Montgomery *et al*, while fully acknowledging the existence of tuberculosis-associated erythema induratum, coined the term 'nodular vasculitis' to describe chronic inflammatory nodules of the legs that showed histopathologic changes similar to those of erythema induratum, that is, vasculitis of the larger vessels and panniculitis.

Erythema induratum and nodular vasculitis had been considered the same disease entity for a long time. However, nodular vasculitis is now considered a multifactorial syndrome of lobular panniculitis in which tuberculosis may or may not be one of a multitude of etiologic components. Therefore, erythema induratum/nodular vasculitis complex is classified into two variants. Erythema induratum of Bazin type and nodular vasculitis or erythema induratum of Whitfield type. Bazin type is related with tuberculous origin, but Whitfield type is not[1].

CASE REPORT

A 14-year male presented with history of fever, weight loss and multiple, painful, hyperpigmented ,firm patches over both legs and dorsum of foot since six months. The patches were measuring 4-5 cms in diameter, irregular in shape erythematous, tender with shallow ulceration . The margins of the lesion were well-defined (Figs. 1 and 2).

FNAC and Biopsy of the lesion were performed.

Cytological Examination

FNAC showed fragments of adipose tissue with a few epithelioid cells and Langhan's type of giant cells and lymphocytes.Cytological diagnosis of granulomatous inflammatory lesion of skin was made (Fig. 3).

Histopathological Examination

Biopsy of the lesion showed skin with inflammatory infiltrate in the deep dermis, composed of a few poorly defined epithelioid granulomas, Langhan's giant cells and mature lymphocytes.No evidence of necrosis was seen. These granulomas

1. Assistant Professor 2. Professor and Head
Department of Pathology, Bangalore Medical College & Research Institute, Bangalore
Correspondence: Dr.C.Nirmala, Assistant Professor, Department of Pathology, Bangalore Medical College and Research Institute, Bangalore. E-mail; chandrannirmala@yahoo.com; Mobile - 9945510250

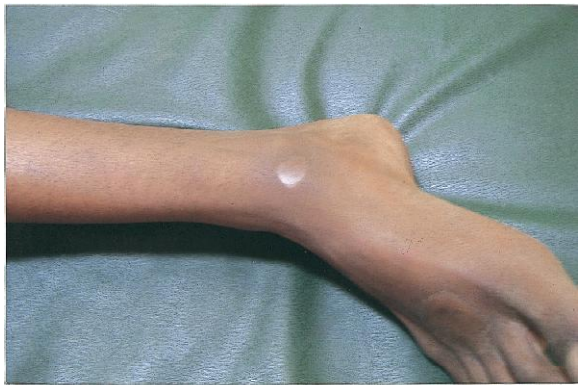

Fig. 1: Gross photograph of the leg lesion 1.

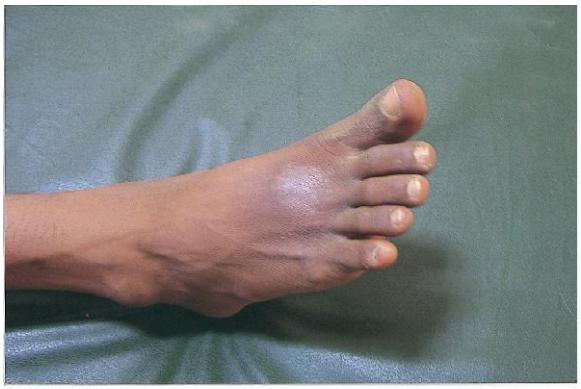

Fig. 2: Gross photograph of the leg lesion 2.

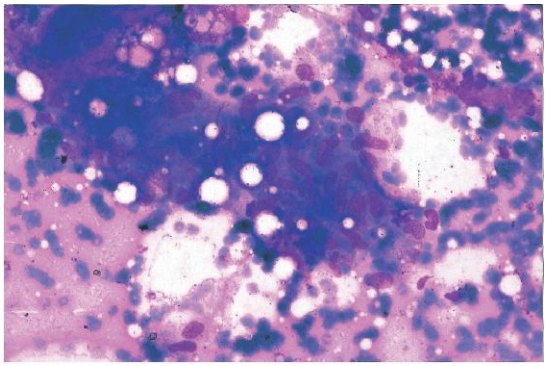

Fig. 3: FNAC smear showing granulomas(giemsa stain).

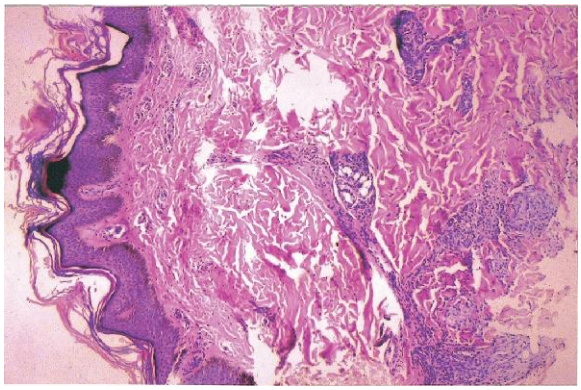

Fig. 4: Biopsy histopathology showing septal granulomatous panniculitis (5x).

showed a septal type of distribution with extensive hyalinization and fibrosis of the surrounding stroma (Figs. 4, 5 and 6). A diagnosis of granulomatous panniculitis with possibility of erythema induratum, a type of cutaneous tuberculosis suggested. The diagnosis was confirmed with other investigations.

Mantoux Test – strongly positive(>2cm dia)

Serological test –TB IgG and IgM antibody levels significantly raised suggesting active tuberculosis.

Treatment

The patient responded well to a course of anti-tuberculous therapy, with marked resolution of the lesions. His fever subsided with significant improvement in the general condition.

DISCUSSION

Erythema induratum is still prevalent in India, Hong Kong, and South Africa. There is a marked female preponderance. The commonest presentation is in a young woman in her 20s but it may present at a later age.The usual site of the lesions is the lower legs (calves, with the shins involved less often) but it may occur in other places too. The trunk, buttocks, thighs, and arms can be involved but this is much rarer. The nodules are usually grouped on the lower third of the legs, especially around the ankles[2].

The nodules are tender and erythematous. The nodules may ulcerate with bluish borders, and cold weather may be the precipitating factor. This produces irregular, shallow ulcers that may cause permanent scarring with hyperpigmentation of the

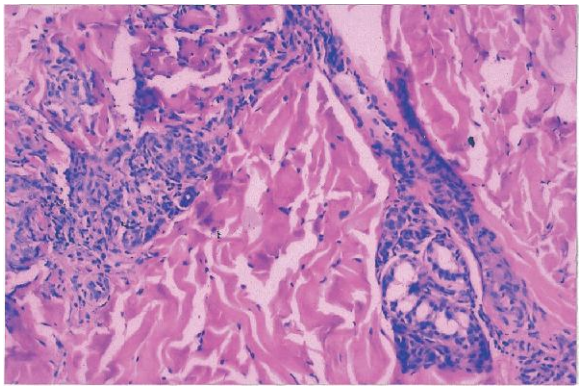

Fig. 5: Histopathology showing septal granulomatous panniculitis (10x).

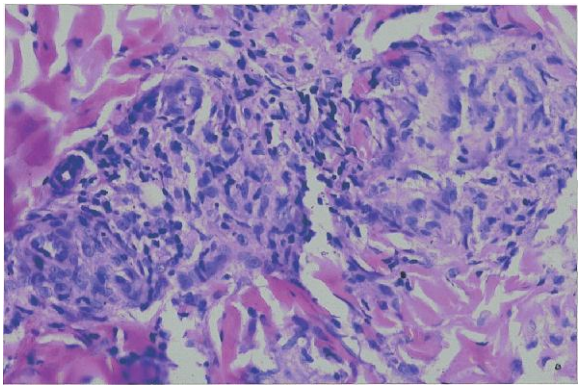

Fig. 6: Histopathology showing septal granulomatous panniculitis (40x)

lesions.They may run a chronic and recurrent course. The legs may be oedematous. About half of patients will give a past or present history of tuberculosis[2].

The disease or diseases represent an inflammatory reaction. Patients with erythema induratum have a strongly positive tuberculin skin test and a marked increase in their peripheral T lymphocyte response to Purified Protein Derivative (PPD) of tuberculin, which is a delayed (type IV) hypersensitivity reaction[1].

A negative Polymerase Chain Reaction (PCR) can be seen in cases of erythema induratum. Only 56%–88% of patients previously diagnosed with cutaneous tuberculosis had a positive PCR result. Schneider *et al* found a positive PCR result in only five of 20 patients with Erythema induratum[5]. Shimizu *et al* failed to isolate *Mycobacterium tuberculosis* by either culturing cutaneous erythema induratum in tissue or inoculating the tissue into guinea pigs[3].

Histopathology varies with duration of lesion. Lobular or septolobular granulomatous panniculitis, restricted to subcutis and lower dermis, involves many contiguous lobules. Inflammatory infiltrate includes neutrophils,lymphocytes, plasma cells, varying combinations of granulomatous inflammation. Granulomas usually poorly developed, with occasional caseation necrosis, vasculitis, focal necrosis. Neutrophils predominate in areas of fat necrosis and septal fibrosis. Vascular changes in small blood vessels show fibrinoid change. In 90% of cases all sizes of arteries and veins comprise endothelial swelling, mixed inflammatory cell infiltrate in wall and periadventitial tissues, sometimes necrotizing vasculitis, particularly in early lesions[2]. The marked septal fibrosis and minimal vascular changes noted in our case corresponds to the long-standing duration of the lesion in our case.

There is considerable controversy in the literature about whether or not vasculitis is a histopathologic requirement to establish the diagnosis of erythema induratum of Bazin. Even accepting vasculitis as a histopathologic criterion, there is no agreement about the nature and size of the involved vessels[4].

In some cases, with all clinico-pathological features of erythema induratum of Bazin, vasculitis could not be demonstrated with serial sections throughout the specimen and, therefore, the presence of vasculitis should not be considered as a criterion *sine qua non* for histopathologic diagnosis of erythema induratum of Bazin[5]. There was no significant evidence of vasulitis noted in our case.

Motswaledi and Schulz1 noted that erythema induratum of Bazin, lichen scrofulosorum, and papulonecrotic tuberculide are the three recognized tuberculides, which are sequelae of

immunologic reactions to hematogenously dispersed antigenic components of *Mycobacterium tuberculosis*[6].

The morphologic, molecular, and clinical data suggest that erythema induratum represents a common inflammatory pathway, that is, a hypersensitivity reaction to endogenous or exogenous antigens. Some have considered EI to be a type III or type IV hypersensitivity reaction to *M. tuberculosis* antigens[7].

CLASSIFICATION OF CUTANEOUS TUBERCULOSIS[6]

Types of cutaneous TB	Features
TB verrucosa cutis	Occurs after direct inoculation of TB into the skin in someone who has been previously infected with mycobacteria Presents as a purplish or brownish-red warty growth • Micro: Hyperkeratosis, acanthosis, acute inflammation with abcess in the upper dermis,mid dermis epithelioid granulomas with central necrosis[8].
Lupus vulgaris	Persistent and progressive form of cutaneous TB Small sharply defined reddish-brown lesions with a gelatinous consistency (called apple-jelly nodules) Micro: Atrophic skin, pseudoepitheliomatous hyperplasia of ulcer margins. Epithelioid granulomas, caseation necrosis minimal or absent[8].
Scrofuloderma	Skin lesions result from direct extension of underlying TB infection of lymph nodes, bone or joints. Micro: Sinus tract lined by epithelioid cells and lymphoid cells[8]
Miliary TB	Skin lesions are small (millet-sized) red spots that develop into ulcers and abscesses. Micro: Micro abcess containing neutrophil cell debris ,surrounded by epithelioid cells and giant cells[8]. Generalised exanthem in patients with moderate or high degree of immunity to TB because of previous infection
Tuberculid	Erythema induratum (Bazin disease) presents as recurring nodules or lumps on the back of the legs (mostly women) that may ulcerate and scar. Micro: Lobular or septolobular panniculitis with granulomas, absence of caseation necrosis, vasculitis(+/-)[5] It is a type of nodular vasculitis. Papulonecrotic tuberculid results in crops of

recurrent crusted skin papules on knees, elbows, buttocks or lower trunk that heal with scarring after about six weeks.

Micro: Leucocytoclastic vasculitis/lymphocytic vasculitis, fibrinoid necrosis and thrombotic occlusion of individual vessels in the centre, epithelioid cells and giant cells in the periphery[8].

Lichen scrofulosorum is an extending eruption of small follicular papules in young persons with underlying TB.

Micro: Superficial dermal granulomas in the vicinity of hair follicles or sweat ducts. Epithelioid granulomas with giant cells and narrow rim of lymphoid cells. Caseation necrosis absent[8].

CONCLUSION

Erythema induratum (Bazin disease) is a tuberculid and is classified under cutaneous tuberculosis.It is considered to be a type 3 or type 4 hypersensitivity reaction to *Mycobacterium tuberculosis* antigens.Vasculitis may not be a significant finding in long standing cases of erythema induratum.

A search for active foci of tuberculosis is to be advocated in all cases of erythema induratum.

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Case Report

TUBERCULOUS EPIDIDYMO-ORCHITIS IN AN UNDESCENDED TESTIS

Punit Tiwari[1]*, Dilip Kumar Pal[2]*, Biplab Kumar Biswas[3] and Mukesh Vijay[1]***

(Received on 24.12.2009; Accepted on 28.1.2010)

Summary: We present an uncommon case of tubercular epididymitis in an undescended testis, diagnosed by Fine Needle Aspiration Cytology (FNAC), which is not reported till now. The treatment is primarily medical with combination of three or four anti-tubercular drugs but sometimes it requires surgical intervention, as in the present case.. ***[Indian J Tuberc 2010; 57:165-167]***

Key words: Epididymo Orchitis. Undescended Testis. Tubercular epididymitis, FNAC.

INTRODUCTION

The incidence of tuberculosis is rising in many parts of the world because of rising HIV prevalence. The genitourinary tract is the most common site for extra pulmonary tuberculosis[1]. Epididymal involvement is rare, may be the most common site[2]. Tubercular infection to the epididymis occurs by downstream from the infected kidneys, but haematogenous spread is another theory for epididymal infection. Clinical presentation combined with imaging procedures like sonography with sterile pyuria raises the suspicion for genito-urinary TB, especially if past history of pulmonary tuberculosis is present. Diagnosis is confirmed by positive cultures, Ziehl Neelsen stain and/or histological examination. We are reporting an unusual case of tubercular epididymo-orchitis in an undescended testis which was never reported before in English literature.

CASE REPORT

A 25-year-old male patient presented with gradually increasing swelling and pain in the left inguinal region since last one year. He had undescended testis in the left side since birth but he did not seek any medical advice for that. On examination, there was a firm tender swelling in the left inguinal region just above the superficial ring. Right-sided testis was within the scrotal sac and of normal size with normal external genitalia.

Laboratory investigations did not show any sign of inflammation. Renal biochemical parameters were within normal limits. X-ray chest suggested old Koch's lesion. Urine in ordinary culture media and in AFB culture was negative. Ultrasonography with 7.5- MHz linear probe suggested swollen heterogeneous epididymis with mostly homogenous echo pattern of the testis with surrounding clear fluid with a hypo-echoic nodule at the bottom of the testis. Sonography of the kidneys and urography were unremarkable. FNAC showed a granulomatous lesion. A tuberculin skin test showed 17 mm induration. Ziehl-Neelsen stain for mycobacteria from three consecutive morning samples of urine were negative. Due to increasing pain and swelling, left inguinal exploration was done and a normal sized testis was found within a sac with a separate nodular growth (2 cm) at the lower part of the sac which was separated from the testis (Fig.1) with gross dilatation of the epididymis. Due to extensive involvement of the epididymis and considering the age of the patient, left-sided epididymo-orchidectomy with removal of part of vas was done. Histopathology of testis showed complete germ cell aplasia without any microscopic evidence of

1. Senior Resident 2. Professor 3. Assistant Professor
* Department of Urology, IPGMER, SSKM Hospital, Kolkata (West Bengal)
** Department of Pathology, Bankura Sammilani Medical College, Kolkata (West Bengal)
Correspondence: Dr. Punit Tiwari, Senior Resident, Department of Urology, IPGMER, Kolkata-700 020

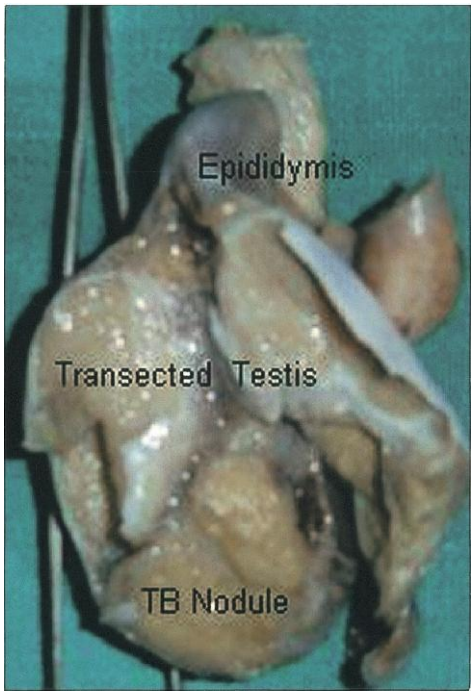

Fig.1: Transected testis and a separate nodule at the bottom in the sac with dilated epididymis.

tuberculosis (Fig. 2A). Histology of the nodule showed scattered caseating granuloma with Langhan's giant cells with fibrous stoma (Fig. 2B). Dilated lumen of the epididymis showed intraluminal tubercular lesion. AFB staining from the nodular tissue and epididymis showed Mycobacterium and AFB tissue culture showed growth of *M. tuberculosis*.

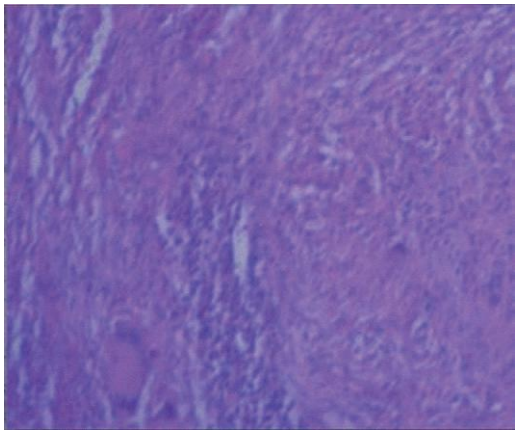

Fig. 2B: Nodule showing caseating granuloma with Langhan's giant

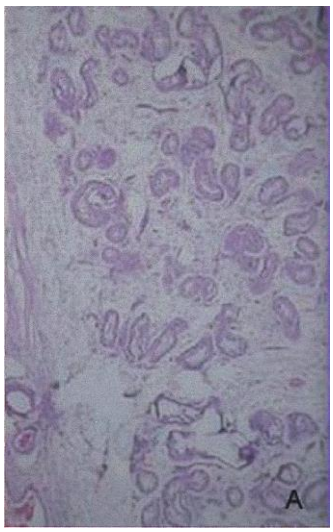

Fig. 2A: Testis showing complete germ cell aplasia (H & E x 10)

Anti-tubercular drugs started in standard doses with rifampicin, INH, ethambutol and pyrazinamide for two months and rifampicin and INH were continued for another four months. The wound was healed with regular dressing and the patient was in good health till two years of follow up.

DISCUSSION

Tuberculosis is a disease which can involve any part of the male reproductive system including the epididymis, vas deference, seminal vesicles, prostate and least commonly the testis[1]. Most commonly involved organ is epididymis in males and fallopian tubes in females. Epididymal involvement usually occurs in young, sexually active men. Usually, the organs are involved by retrograde spread of infection from the urinary bladder[1,3]. The epididymis can also be infected through the haematogenous spread due to high vascularity of the globus minor[2] as in the present case, where we could not find any other genito-urinary organ involvement by tuberculosis. Usually, the patients present with scrotal pain, tenderness and swelling[3]. Sometimes it is a cause of male infertility[4]. The most common presentation is scrotal swelling and most common sign is scrotal tenderness[5]. Sometimes it may present with scrotal or testicular mass or abscess. Involvement is usually unilateral but bilateral involvement is also reported[5].

A positive tuberculin test supports TB infection but a negative test does not rule it out[4]. Scrotal ultrasonography suggests a gross enlargement of epididymis with marked heterogeneous echo texture[1,4]. Though Polymerase Chain Reaction (PCR) facilitates and accelerates the diagnostic specificity and sensitivity of 98% and 95% respectively[6] but it was not done as the facility is not available in our institution. In some studies, FNAC confirmed the diagnosis of epididymal tuberculosis in 80% cases[5] as in the present case.

A definite diagnosis depends upon positive culture, Ziehl-Neelsen staining and FNAC/ histological examination of the suspected tissue[4]. However most investigators suggest PCR in combination with cultures and Ziehl-Neelsen staining and FNAC from the palpable mass for diagnosis of genito-urinary TB and for a definite treatment plan[4,5].

Treatment consists of combination of four drugs, i.e. rifampicin, INH, ethambutol and pyrazinamide as have been applied in the present case. The duration of treatment is now reduced to 6 to 9 months if the primary drug resistance is ruled out. Though some authors suggest that medical therapy is the treatment of choice[1,4] yet indications of surgery are extensive epididymal and testicular involvement, abscess formation in between epididymis and testis, if the mass cannot be distinguished from a testicular tumor and when anti-tubercular chemotherapy is found to be resistant. **Epididymectomy with or without orchidectomy is the treatment of choice if chemotherapy fails or extensive disease is found[4].**

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Indian J Tuberc 2010; 57:168-170

STATUS REPORT ON RNTCP*

RNTCP has continued to achieve the twin objectives of NSP case detection and treatment success rate at the national level during the first quarter, 2010. With this, it is evident that the programme, while consolidating and sustaining its past achievements, is progressing satisfactorily towards achieving the TB related Millennium Development Goals.

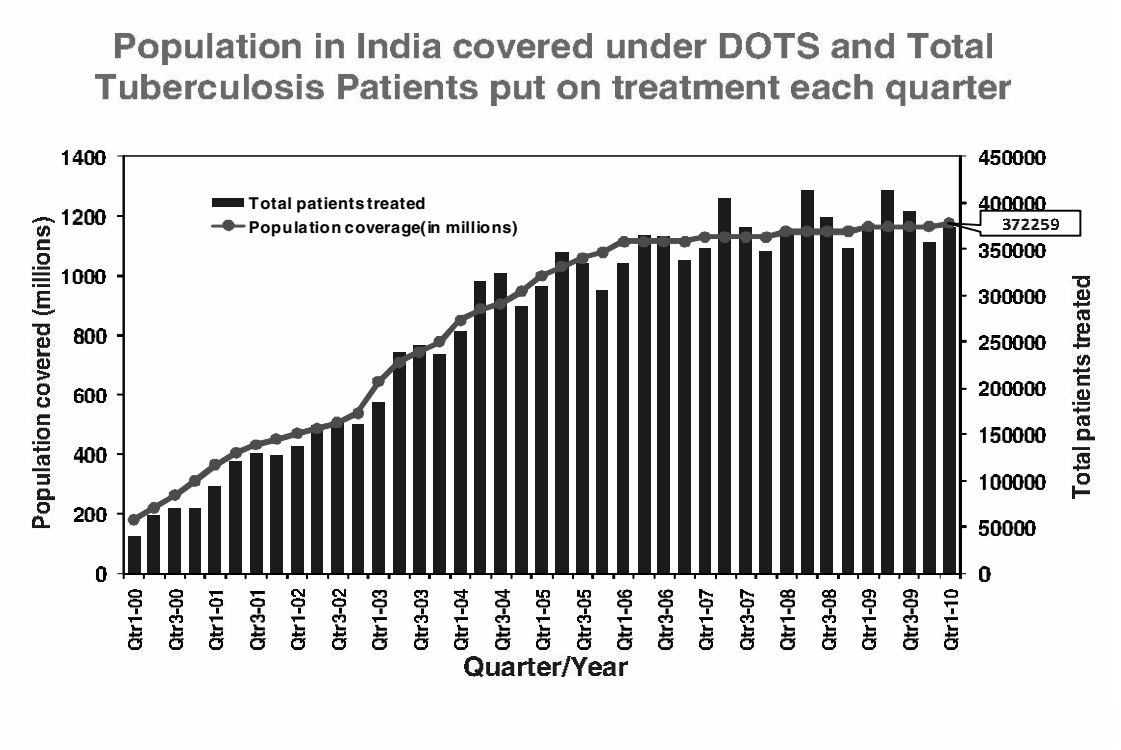
RNTCP performance in first quarter 2010

During the quarter, over 1.84 million suspects were examined, 230,270 sputum positive cases were diagnosed, and 372,259 TB cases were registered for treatment. The annualized total case detection rate is 127 cases per 100,000 population. With a total of 153,471 new smear positive cases being registered for treatment, the new smear positive TB case detection rate (annualized) for the first quarter 2010 was 70%. In addition to this, 90,809 new smear negative cases, 57,293 new extra-pulmonary cases, 48,111 smear positive re-treatment cases and 22,317 re-treatment Others' were also registered for treatment in this quarter. The treatment success rate amongst the new smear positive PTB cases registered in the first quarter 2009 was 87% and the sputum conversion rate of patients registered during fourth quarter, 2009 was 90%. The default rates among NSP (5.5%), NSN (6.9%) and re-treatment cases (14.1%) continue to show the declining trend over the past several quarters.

Major activities during the quarter

Programme review

RNTCP was reviewed in detail by Secretary H&FW on 15th January 2010 with Health Secretaries,



* Dr. L. S Chauhan, DDG (TB), Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India, New Delhi

Table: Performance of RNTCP Case Detection (2010 first quarter), Smear Conversion (2009, fourth quarter), and Treatment Outcomes (2009, first 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 detection rate	New smear positive patients registered for treatment	Annualized new smear positive case detection rate (%)		No of new smear negative cases registered for treatment	No of new EP cases registered for treament	No. of smear positive retreatment cases registered for treatment	3 month conversion rate of new smear positive patients	Cure rate of new smear positive patients	Success rate of new smear positive patients
Andaman & Nicobar	5	189	93	190	158	65	54	72%	49	58	16	96%	91%	91%
Andhra Pradesh	840	163	19548	28340	135	12261	58	78%	7396	3252	3939	92%	87%	89%
Arunachal Pradesh	12	193	262	510	166	172	56	75%	139	68	82	92%	85%	86%
Assam	302	121	5641	9637	128	4100	54	72%	2703	1206	878	88%	83%	85%
Bihar	964	97	11618	19758	82	8789	36	49%	6164	1166	1861	88%	82%	90%
Chandigarh	14	297	547	650	190	220	64	68%	106	198	87	89%	87%	88%
Chhatisgarh	239	113	3476	6977	117	2803	47	59%	2547	875	431	89%	82%	87%
D & N Haveli	3	167	69	98	116	33	39	49%	23	13	19	94%	85%	85%
Daman & Diu	3	234	54	87	134	21	32	41%	19	19	13	62%	54%	63%
Delhi	179	241	6416	13021	290	3568	80	84%	2267	4165	1799	91%	86%	86%
Goa	17	220	297	552	129	188	44	55%	109	151	62	96%	83%	83%
Gujarat	582	179	15204	19436	134	8732	60	75%	2329	2763	3986	92%	87%	87%
Haryana	250	164	5875	8831	141	3257	52	55%	1710	1511	1768	90%	85%	85%
Himachal Pradesh	67	262	2241	3660	218	1360	81	85%	638	800	625	93%	86%	88%
Jammu & Kashmir	116	218	2428	3636	126	1798	62	65%	533	754	464	93%	90%	91%
Jharkhand	310	113	5485	9316	120	4278	55	74%	2976	663	703	91%	85%	89%
Karnataka	588	195	10866	16690	114	6460	44	59%	3666	3243	2322	87%	80%	82%
Kerala	343	267	3952	6885	80	2834	33	66%	1654	1586	588	83%	83%	84%
Lakshadweep	1	87	2	3	16	2	11	14%	1	0	0	#DIV/0!	100%	100%
Madhya Pradesh	711	113	12170	20394	115	7810	44	55%	6370	2402	2498	90%	85%	87%
Maharashtra	1111	156	19717	34905	126	13206	48	59%	7783	6671	4051	90%	84%	85%
Manipur	24	135	314	807	133	233	38	51%	256	166	62	88%	85%	86%
Meghalaya	26	198	608	1108	171	377	58	78%	244	224	150	83%	80%	81%
Mizoram	10	198	203	623	251	140	56	75%	153	178	64	90%	91%	92%
Nagaland	22	155	468	893	161	318	57	76%	219	170	98	93%	89%	89%
Orissa	404	131	7557	12492	124	5592	55	65%	2920	2351	1012	89%	83%	86%
Puducherry	13	419	632	347	104	145	44	58%	71	72	52	90%	85%	85%
Punjab	274	157	5674	9607	140	4139	60	64%	1663	1901	1527	91%	85%	87%
Rajasthan	668	135	15345	26567	159	9506	57	71%	7676	3486	4716	92%	87%	88%
Sikkim	6	273	209	384	254	135	89	119%	69	90	63	92%	87%	87%
Tamil Nadu	670	228	11844	20750	124	8343	50	66%	5463	4105	2172	91%	86%	87%
Tripura	36	151	444	672	75	370	41	55%	124	98	56	91%	88%	88%
Uttar Pradesh	1973	141	41690	65268	132	29171	59	62%	17067	7929	8233	92%	85%	88%
Uttarakhand	98	177	2441	3592	147	1323	54	57%	822	630	621	89%	81%	85%
West Bengal	887	170	16880	25573	115	11722	53	71%	4880	4329	3093	89%	84%	85%
Grand Total	**11767**	**157**	**230270**	**372259**	**127**	**153471**	**52**	**70%**	**90809**	**57293**	**48111**	**90%**	**85%**	**87%**

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

NRHM Directors and Directors of Health Services of 17 states. The programme was reviewed by Joint Secretary (PH) and DDG(TB) with State TB Officers and Directors of Health Services on 14th January. Detailed review of the programme was also done during the State TB Officers and Consultants' meeting in last week of January 2010. The major thrust given in all these reviews is on the importance of Universal access for TB care. Every TB patient in the country should have access to early case detection and standardized care in all health care facilities. Strategies for the universal access for TB care were developed in the meeting.

Progress in Supervision, Monitoring and Training

Two central internal evaluations and 25 state internal evaluations were conducted in first quarter 2010.

Electronic Monitoring system in RNTCP has been upgraded with a new software, window based EPICENTRE 2006. All districts in the country have upgraded to this system from first quarter 2010. The RNTCP website tbcindia.org has been updated with addition of two new tabs for TB/HIV and DOTS-Plus.

The RNTCP modules for programme managers have been revised and are being piloted.

Progress in accreditation of Intermediate Reference Laboratories (IRL)

RNTCP is in the process of establishing a network of about 27 Intermediate Reference Laboratories (IRL), and 16 other labs across the country in a phased manner for diagnosis and follow up of MDR TB patients. 13 labs including three in private sector have been accredited. Accreditation processes are in final stages in another four labs.

Progress in the DOTS- Plus services for MDR TB cases

DOTS Plus services for management of MDR TB are now available in 125 districts covering a population of 260 million in 10 states. Till date, a total of around 1806 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 procurement and drug logistics management

Drug logistics management training was conducted for RNTCP Consultants in January 2010.

Progress in TB HIV Collaborative Activities

Training of trainers completed for programme managers for implementing intensified package for TB-HIV in the states of Kerala and Punjab and the UT of Chandigarh. Joint TB/HIV review was conducted at National level on 29th January 2010. Training on HIV/TB for Nodal officers and consultants of Intensified Package and ART modules was conducted at NTI Bangalore in February 2010. National Technical Working Group Meeting on HIV- TB Collaborative activities was held on 23rd March 2010.

ABSTRACTS

Tuberculosis in Diabetics: Features in an endemic area

Dursun Tatar, Gunes Senol1, Serpil Alptekin, Caglar Karakurum, Mert Aydin, and Ipek Coskunol. *Jpn J Infect Dis* 2009; **62:** 423-27

Diabetes mellitus (DM) is known as one of the factors that increases the risk of tuberculosis (TB). TB can also show atypical clinical presentation and localization in diabetics. The aim of the study was to evaluate the features of TB in diabetics in our region. Between 1997 and 2003, all cases of diabetic TB patients and an equal number of non-diabetics treated and followed at the Esrefpasa Tuberculosis Dispensary were analyzed retrospectively. A total of 78 (7.3%) TB cases in DM patients was encountered among 1,063 TB cases. Cavity formation and atypical localization were more often found in diabetics ($P < 0.05$). Duration of treatment was longer in diabetics ($P < 0.05$). The rate of drug resistance was higher in DM cases, but cure rates were similar between groups. A diagnosis of TB should be considered in diabetics with an abnormal chest radiograph, in the presence or absence of specific clinical symptoms, in endemic regions. Diabetic TB cases should be followed, especially closely in terms of cure time and drug resistance.

Cost-effectiveness of QuantiFERON®-TB test vs. tuberculin skin test in the diagnosis of latent tuberculosis infection.

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Diagnostic accuracy of the microscopic observation drug susceptibility assay; A pilot study from India

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The microscopic observation drug susceptibility (MODS) assay is a rapid, sensitive, low-cost liquid culture technique. The objective was to establish the accuracy of MODS for the detection of active pulmonary tuberculosis (TB), and to document the costs and challenges of setting up this assay in a low-income setting. This prospective blinded pilot study of 200 adult TB suspects was conducted at a tertiary referral hospital in India. Reference standard included culture (Lowenstein-

Jensen and automated liquid culture) and clinical diagnosis. Patients were mostly males *(n* = 122, 61.1%) and out-patients *(n* = 184, 92.0%), with a mean age of 40.4 years (standard deviation 16.2). Seventeen (8.5%) were human immunodeficiency virus infected and 47 (23.5 %) were reference culture-positive. Compared to reference culture, MODS was 78.9% sensitive (95% CI 62.2-90.0) and 96.7% specific (95% CI 92.0-98.8). Clinical assessment suggested that MODS was false-negative in 3/8 reference culture-positive MODS-negatives and true-positive in 4/6 reference culture-negative MODS-positives. MODS was faster than solid (P < 0.001) and liquid culture (P = 0.088), and cheaper than both. MODS may be a good alternative to automated liquid culture, but there were several challenges in setting up the assay. Prior training and validation, setup costs and inability to rule out cross-contamination need to be taken into account before the test can be established.

Predictors and mortality associated with treatment default in pulmonary tuberculosis.

K. Kliiman and A. Altraja. *Int J Tuberc Lung Dis* 2010; **14(4):** 454-63

The objective was to identify risk factors for default from pulmonary tuberculosis (TB) treatment and to assess mortality associated with default in Estonia. All patients with culture-confirmed pulmonary TB who started treatment during 2003-2005 were included in a retrospective cohort study. In 1107 eligible patients, the treatment success rate was 81.5% and the default rate 9.4% (respectively 60.4% and 17.0% in multi-drug-resistant TB [MDR- TB]). Independent predictors of treatment default were alcohol abuse (OR 3.22, 95% CI 1.93-5.38), unemployment (OR 3.05, 95% CI 1.84-5.03), MDR-TB (OR 2.17, 95% CI 1.35-3.50), urban residence (OR 1.85, 95% CI~ 1.00-3.42) and previous incarceration (OR 1.78, 95% CI 1.05-3.03). Of the defaulters, 29.4% died during follow up (median survival 342.0 days). Cox regression analysis revealed that unemployment was associated with all-cause and TB-related mortality among defaulters (respectively HR 4.58, 95% CI 1.05-20.1 and HR 11.2, 95% CI 1.58-80.2). HIV infection (HR 51.2, 95% CI 6.06-432), sputum smear positivity (HR 9.59, 95% CI 1.79-51.4), MDR-TB (HR 8.56, 95% CI 1.81-40.4) and previous TB (HR 5.15, 95% CI 1.64-16.2) were predictors of TB-related mortality. The main risk factors for treatment default can be influenced. Interventions to reduce default should therefore concentrate on socially disadvantaged patients and prevention of alcohol abuse, with special attention given to MDR-TB patients.

Can hepatitis B virus infection predict tuberculosis treatment liver toxicity? Development of a preliminary prediction rule.

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Liver toxicity due to tuberculosis (TB) treatment is a frequent cause of treatment interruption, and may sometimes lead to a change in therapy to a less potent regimen. The objective was to estimate the risk of hepatotoxicity in patients with or without hepatitis B virus (HBV) infection receiving TB treatment and to develop a clinical prediction rule. A prospective observational follow-up was conducted. Data from 154 patients who underwent TB treatment were analysed. Crude risk ratios were estimated and a Cox proportional hazards model was fit. The mean follow-up time was 187 days. Crude risk ratios showed that ethnicity, human immuno-deficiency virus infection, multiple sexual partners, highly active antiretroviral treatment, and clinical forms of TB were possible predictors of liver toxicity. HBV infection and other sexually transmitted diseases showed considerable relative risk, although not statistically significant. The Cox proportional hazards model identified the following predictors of hepatotoxicity: White ethnicity, multiple sexual partners, high baseline alanine transferase and clinical forms of TB. Active HBV, indicated by the detection of surface antigen HBV, could predict hepatotoxicity, although with low precision. Using this information, we were able to apply a score and draw a nomogram to estimate survival probabilities and median times to event for each patient.

ABSTRACTS

Tuberculosis in Diabetics: Features in an endemic area

Dursun Tatar, Gunes Senol1, Serpil Alptekin, Caglar Karakurum, Mert Aydin, and Ipek Coskunol. *Jpn J Infect Dis* 2009; **62:** 423-27

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GUIDELINES FOR CONTRIBUTORS

GENERAL

The *Indian Journal of Tuberculosis (IJT)* is published four times in a year; January, April, July and October. It publishes original articles on tuberculosis, respiratory diseases, case reports, review articles, and abstracts of articles published in other medical journals and book reviews. Every issue contains editorial, sections on contemporary subjects, radiology forum and a forum for readers to express their opinions on published articles and raise questions on subjects appearing in the journal.

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All correspondence relating to the *IJT* should be addressed to: *The Editor, Indian Journal of Tuberculosis,* Tuberculosis Association of India, 3 Red Cross Road, New Delhi - 110 001.

Articles are published on the understanding that every author confirms his participation in the study concerned and approves its content, and an affirmation that the article is original and has not been published/submitted for publication elsewhere and will not be so submitted, if accepted for publication in the *IJT.* A letter to this effect signed by the author should accompany the article.

All received articles are published, if found suitable, after completion of basic formalities. Notification of acceptance or rejection will be sent within three months of receipt. The decision of the Editor is final who reserves the right to make editorial corrections.

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Three copies of the manuscripts, including diagrams and photographs, typed on one side of the page with double spacing and wide margins should be submitted. To facilitate referral, it would be appreciated if compact diskettes are also enclosed. The preferred package is MS Word. The author should mention e-mail address, telephone and fax numbers apart from complete postal address with PIN code. Articles can also be sent by e-mail at tbassnindia@yahoo.co.in.

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This should contain: (1) A concise informative title; (2) The name of the principa1 author followed by names of other authors without giving qualification or position held, except numera1 on top of last letter of name; (3) A running title usually not exceeding five words; (4) A word count of the text, excluding references, tables and figures; (5) In the case of original articles, a few key words for indexing purposes, using where possible, terms of medical subjects headings list from index medicus. The position held by each author in any institution should be indicated at the bottom of the title page along with the name and address of the author to whom correspondence regarding the manuscript has to be sent. Fax and telephone numbers (both landline and mobile) and e-mail ID should also be given.

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An informative summary of not more than 250 words should be provided that can be understood without reference to the text (see Ann Intern Med 1990; 113: 69-76). The summary should be as per Vancouver format as follows: Background, Aims, Methods, Results and Conclusions. Unstructured

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l. Research and experimental manuscripts should follow the usual conventions, as fo11ows:

Introduction: Setting forth clearly the aim of the study or the main hypothesis, with reference to previous studies and indicating the method used.
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Discussion should be related to the aims, objects and results of the study.

Care should be taken that language is grammatically correct and fluent, that all relevant information is included, irrelevant details omitted and repetitions, especially from section to section, avoided.

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Book References to a piece of work (book or monograph) should include the authors' names, the title of the piece of work, the place and year of publication:

e.g. Crofton, J. and Douglas, A. *Respiratory Diseases,* 1st Edition. Edinburgh: Blackwell Scientific Publications Ltd, 1969.

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RS, Muller NL, Colman N, Pare PD, Bralow L, ed Fraser and Pare's *Diagnosis of Diseases of Chest;* 4th Ed; Vol III. Philadephia: W.B. Saunders Co, 1999: pp 2021-48.

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Acknowledgements

Acknowledgements should be brief (not more than six lines). Acknowledge only those persons who made substantial contribution to the study and all sources of support in the form of grants.

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