Registered with the Registrar of Newspapers of India under No. 655/57

Indian Journal of Tuberculosis

Published quarterly by the Tuberculosis Association of India

Vol. 58 : No. 3 **July 2011**

Subscription

Inland	
Annual	Rs.800
Single Copy	Rs.200
Foreign	
For SAARC countries	US $ 30
For South East Asian and Eastern countries	US $ 35
For other countries	US $ 40

Cheques/D.Ds. should be drawn in favour of "Tuberculosis Association of India, New Delhi"

Contents

Bibliographic details of the journal available in ICMR-NIC Centre's IndMED data base (http://indmed.nic.in). Full-text of articles from 2000 onwards are available online in medIND data base (http://medind.nic.in). ***IJT*** **is indexed in MEDLINE of National Library of Medicine, USA.**

Published and printed by Tejinder S. Ahluwalia, on behalf of the Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110001 Phone: 011-23711303; 23715217 and printed at Cambridge Printing Works, B-85, Naraina Industrial Area-II, New Delhi-110 028 Phone : 45178975.

Indian Journal of Tuberculosis

Vol.58 **New Delhi, July 2011** **No.3**

Editorial

TUBERCULOSIS MANAGEMENT-TIME FOR PARADIGM SHIFT?

[*Indian J Tuberc 2011; 58: 97-101*]

World Health Organisation (WHO) has published the fourth edition of *Treatment of tuberculosis:guidelines*.[1]It extensively covers various aspects of management of TB including emerging problems such as multidrug resistant-tuberculosis (MDR-TB) and HIV-TB co-infection.The inherent strength of the guidelines is the simplified manner of presentation, greatly benefitting readers to understand the key messages. There are many recommendations, which call for a radical change in existing treatment practices throughout the world. Majority of these recommendations are not based on sound evidence and are derived from common consensus, affected by individual believes and perception. Hence, these recommendations are not binding on the countries that choose not to implement them. Table 1 summarises salient recommendations and reasons to approve or disapprove the same.

There are potential areas of disagreement in the guidelines, requiring re-evaluation and further strengthening.Majority of the members constituting guidelines' group belong to countries with minority of TB cases and thus have limited experience of treating these patients. Besides, since most of these countries are rich in resources, respective authors may be unaware of challenges faced by resource constrained developing nations in executing the desired recommendations in a programmatic set up. There is minimal representation from South-East Asian countries that account for one third of global TB burden.[2]More participation from this part of the world would have facilitated decision making in areas of conflicts. At the same time, it would have addressed operational difficulties and ground realities in implementing the recommendations, thereby ensuring universal acceptance of the document.

In the new definition for definite case of TB, a patient with even one positive AFB smear is considered as a 'case' in countries having proper functional external quality assurance (EQA) system. The number of specimens has been reduced from three to two for screening of TB suspects, since the additional yield of third sputum smear is low (2 to 5%).[3]This approach decreases burden on laboratories and simultaneously helps in early initiation of treatment. Moreover, number of visits for collection of sample are reduced, thereby more convenient for the patients. However, this applies only when there is well functioning EQA with blind rechecking as well as good internal quality control helping to decrease false positive results. This is a significant limitation for resource limited settings, where ensuring quality control is not always feasible. Hence, this recommendation, if implemented blindly, may lead to transmission of disease, propagation of drug resistance, increased mortality and loss of confidence of community in the programme.[4]Another situation necessitating consideration here is HIV-TB co-infection. As the degree of immunosuppression worsens in these patients, cases with paucibacillary sputum samples begin to increase, who also have atypical findings on chest X-ray.[5] Reducing number of sputum samples for screening of tuberculosis in such patients will delay the diagnosis, miss the potential cases and their treatment. Thus, the suitability of this recommendation needs to be evaluated before applying it in HIV-TB co-infected population.

WHO has strongly recommended daily therapy in both HIV positive as well as negative population as opposed to conventional thrice weekly regimen. It may be a good suggestion in HIV positive patients,

Table 1: Summary of comments on individual recommendations

RECOMMENDATIONS	COMMENTS
RECOMMENDATIONS SUPPORTED	
A)**Recommendations for diagnosis**	
1) Discontinuation of use of categories to classify patients	Helps to improve priority status of MDR-TB
2) Discontinuation of using course of antibiotics to help in diagnosis of TB in HIV/AIDS patients.	Strongly supported; This recommendation will prevent delay in diagnosis and treatment initiation.
3) Use of 2 sputum samples instead of conventional 3 samples to screen for TB suspects	Applicable to settings with well functioning EQA as well as good internal quality control; significant limitation in resource constrained settings, where ensuring quality control may not be possible; caution in HIV- TB patients where higher number of paucibacillary cases
B)**Recommendations for treatment and follow-up**	
1) Treatment with SCC using 6 months of rifampicin	Already followed in national programmes in high burden countries (e.g. RNTCP in India);sufficient evidence to show unequivocal efficacy
2) Referral of end-IP sputum smear positive patients for DST	Useful in present circumstances but may not be operationally feasible everywhere, as many countries still lack desired infrastructure and resources to carry out DST on routine basis
3) Carrying out DST for previously-treated patients, and using rapid DST results to guide treatment regimen	
RECOMMENDATIONS REQUIRING MORE EVIDENCE	
1) Daily therapy in HIV-TB co-infected patients	Good suggestion, but paucity of sufficient evidence; trials with large sample size required to document clear cut benefits
2) Addition of ethambutol in continuation phase	Level of isoniazid resistance where this needs to be applied remains unknown; Increased risk of ocular toxicity (dose and duration dependent)[10]; Increased pill burden; Increased risk of acquired ethambutol resistance-deleterious for management of MDR-TB regimen
RECOMMENDATIONS NOT ACCEPTABLE	
1) Daily therapy in non- HIV patients	Success of DOTS establishes efficacy of intermittent regimen, high cure rates in India and China using intermittent therapy (85% and 90% respectively),[7]; Operational limitations of daily treatment; Lower incidence of certain important side-effects with intermittent treatment (hepatotoxicity, arthralgia)
2) Abandoning extension of IP phase in end-IP sputum positive patients	Study from Bangladesh reveals low relapse rates with IP-extension; No strong evidence showing benefit of discontinuation of IP extension
3) Isoniazid prophylaxis in household contacts and people living with HIV/AIDS who do not have active TB (irrespective of their HIV status)	Not suitable in country like India due to high reinfection rates and increasing INH resistance[11], with better ART services and lowering of threshold for initiating treatment, patient may not develop TB at all; lack of resources in developing countries
4) Empirical treatment for MDR-TB in patients with treatment failure/patients with high likelihood of MDR-TB	Chance of subjectivity in treatment decision; increased cost of treatment; limited capacity in high burden countries to treat MDR-TB
5) Inclusion of high dose INH for treatment of MDR-TB under programmatic setup	Requirement for facility to detect level of INH resistance, inadequate data on safety profile

SCC: Short Course Chemotherapy;DST: Drug Susceptibility Testing

where small clinical trials and studies have consistently shown better cure rates, lower frequencies of relapse and treatment failure with daily treatment.[6] Nevertheless, there is paucity of well-designed and adequately powered randomized trials, sufficiently addressing this problem in HIV-TB co-infection. The extension of this guideline to include HIV negative population should not have a blanket approach. All over the world, Directly Observed Treatment Short-Course (DOTS) strategy has already shown dramatic improvement in cure rates, establishing the efficacy of intermittent therapy. Data from India and China, two high burden countries with maximum number of tuberculosis patients have shown success rate* of treatment to be more than 85 % and 90% respectively with intermittent therapy.[7] Also, WHO report 2010 has shown that globally, the rate of treatment success for new sputum smear-positive cases of pulmonary TB, who were treated in the 2008 cohort was 86%, with improving trends.[7] No further evidence is required to prove effectiveness, tolerability and feasibility of intermittent regimen under a programmatic set up. In addition, numerous other reasons favour thrice weekly treatment. First, certain adverse effects (e.g. hepatotoxicity, arthralgia), are lower in alternate day regimen.[8,9] This is significant in Revised National Tuberculosis Control Programme's (RNTCP) decentralized treatment, where not every patient is under expert medical care. Second, beneficial results for daily therapy may not get converted into actual benefits in a programme, where individual treatment provider may choose to opt out due to increased work load. Third, high dropout rate is expected with daily treatment in countries like India, where patients have to travel long distances in villages to procure single dose of ATT. Considering these factors, it can be said that advantages of daily therapy may not be worth its risk. Therefore, further evidence is needed to document its clear cut benefit, and till that time, thrice weekly regimen remains 'acceptable' in a programmatic set up.

The guidelines also recommend addition of ethambutol in continuation phase in areas of high level of isoniazid resistance. This is a good suggestion; nonetheless, many obstacles need to be cleared before its implementation. First, the threshold of community INH resistance where this policy should be considered remains unknown. Second, though ethambutol is relatively free of side-effects, yet adding it in continuation phase would definitely increase risk of irreversible ocular side-effect.[10] As shown by observational studies, this is a duration dependent side effect and thus incidence is expected to increase many folds after its inclusion in Continuation Phase (CP).[10] Third, as mentioned in guidelines itself, there is inadequate evidence about ability of ethambutol to "protect rifampicin" in patients with pre-treatment isoniazid resistance. Fourth, addition of ethambutol implies increasing pill burden, which may be unnecessary in 82-85% of cases (in India, where the INH resistant rate is approx. 15-18%).[11,12] Last but not the least, chances of acquired resistance to ethambutol will increase after its inclusion for longer duration of treatment, adversely affecting treatment options for MDR-TB. Therefore, despite moderate INH resistance prevalent in India, the risk- benefit of adding ethambutol should be weighed before implementing this recommendation.[11, 12]

A short course of antibiotics is no longer recommended to aid in diagnosis of tuberculosis in sputum smear negative HIV-TB co-infected patients. This approach will help to reduce delay in treatment initiation, consequently decreasing morbidity and unnecessary cost of antibiotics. Thus, this recommendation is strongly supported and should be executed without hesitation.

Extra-pulmonary tuberculosis (EPTB) in patients with HIV/AIDS should have received greater emphasis. EPTB has higher incidence in retrovirus positive patients and usually portends poor prognosis. Diagnosis is usually based on the imaging studies due to difficulties in obtaining tissue diagnosis. This constitutes a significant limitation in developing countries, where such facilities may not be available.

* The success rate of treatment includes the percentage of new smear-positive patients who are cured (i.e., whose sputum smear is negative) plus the percentage who complete treatment without bacteriologic confirmation of cure

This, coupled with hazards of radiation exposure and need to undergo these investigations repeatedly for follow-up, makes them unsuitable for use in a programme. Another grey area in management of HIV-TB co-infection is the duration of treatment for both pulmonary as well as extra-pulmonary cases. Some authorities recommend extended duration of treatment whereas others do not. The reasons cited for not advocating extended duration of treatment are operational difficulties, stigmatisation of patients by separate regimen, drug interaction of rifampicin and a greater chance of acquired rifampicin resistance. However, one has to keep in mind the fact that HIV-TB co-infected patients are significantly more prone to relapse with higher case fatality rates.[13-15] Studies with longer duration of treatment (~8 months) have shown relapse rate to be much lower.[13]There is a need for guidelines to address these issues of diagnostic uncertainties in EPTB and conflicts regarding duration of treatment, documentation of cure and outcome in HIV-TB co-infection.

The guidelines further recommend discontinuation of extension of intensive phase (IP) for patients having positive sputum smear at the end of second month of treatment. No concrete evidence has been provided in support of this statement. Guidelines have mentioned one study, currently underway in Bangladesh, where preliminary results have revealed significantly lower relapse rate in patients with extension arm. Any increase in relapse rate will be deleterious in countries like India, where relapse notification rate is quite low. Besides, extending intensive phase promotes adherence to sputum monitoring, which is of great importance in identifying MDR suspects. End-IP extension is operationally feasible and there is no significant cost benefit achieved by its discontinuation. Hence, it should be continued without interruption.

Empirical treatment for MDR-TB in patients with treatment failure and other sub-groups with high likelihood of MDR-TB has been advocated by the current guideline. This recommendation has potential to introduce subjectivities in treatment decisions, unnecessarily exposing some patients to higher pill burden, undesirable side-effects of second-line drugs and *inconvenience* of taking injectables on daily basis. Moreover, it may lead to indiscriminatory use of second-line drugs, further contributing to the cost of therapy and drug resistance.

High dose isoniazid (INH) has been included as one of the options for treatment of MDR-TB. One requires facility to detect level of INH resistance before using this drug in higher doses. This is difficult in a programmatic setup, where number of samples becomes considerably large. Besides, safety profile of high dose isoniazid has not been studied adequately. These factors, along with limited resources in the high burden countries, make it a poor choice for treatment of MDR TB. Hence, use of high dose INH should be reconsidered in a programmatic set up and may be practised only as part of individualised therapy.

Isoniazid prophylaxis has been advocated in household contacts and people living with HIV/AIDS, who do not have active TB (irrespective of their HIV status). This is not suitable for application in countries like India, where chances of reinfection are high, nullifying any advantages gained by prophylaxis. Increasing isoniazid resistance will further decrease its effectiveness. More importantly, now more and more patients with HIV are initiated treatment at higher CD4 counts (less than 350 cells/µl), which decreases the probability of developing active tuberculosis. In resource constraint developing countries, the first priority should always be treatment of active disease with regular drug supply and quality medications.

Overall, the fourth edition has dealt with various problems of tuberculosis including drug resistance effectively. However, certain areas lack adequate evidence to implement the recommendations

as desired. There is immense requirement for clinical trials with good study design and large sample size to generate more evidence in order to rationalize treatment in all controversial areas. It is hoped that for the next edition, there will be wider representation from high burden countries, data based on sound evidence and recommendations to take care of existing conflicts in TB management. This will help to reduce mortality, morbidity and economic losses due to this disease, thereby improving TB scenario all over the world.

Priya Tiwari, Manish Soneja and Surendra K. Sharma
Department of Medicine
All India Institute of Medical Sciences
New Delhi
Telephone: 911126593303 (O), 911126594415 (O);
Fax: 911126589898;
Email: sksharma.aiims@gmail.com, sksharma.aiims@yahoo.com

REFERENCES

1. World Health Organization. Treatment of tuberculosis. Guidelines for National Programmes. 4th ed. WHO/HTM/TB/2009.420. Geneva: World Health Organization, 2010.
2. Tuberculosis in the WHO South-East Asia Region. *Bulletin of the World Health Organization* 2010; **88**:164.
3. Mase SR, Ramsay A, Ng V, Henry M, Hopewell PC, Cunningham J et al. Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. *Int J Tuberc Lung Dis* 2007; **11(5)**: 485-95.
4. Nguyen TN, Wells CD, Binkin NJ, Pham DL, Nguyen VC. The importance of quality control of sputum smears microscopy: the effect of reading errors on treatment decisions and outcomes. *Int J Tuberc Lung Dis* 1999; **3**: 483-7.
5. Elliott AM, Hayes RJ, Halwiindi B, Luo N, Tembo G, Pobee JOM et al. The impact of HIV on infectiousness of pulmonary tuberculosis: a community study in Zambia. *AIDS* 1993; **7**: 981-7.
6. Khan FA, Minion J, Pai M, Royce S, Burman W, Harries AD, *et al.* Treatment of active tuberculosis in HIV-coinfected patients: a systematic review and meta-analysis. *Clin Infect Dis* 2010; **50**: 1288-99.
7. World Health Organization. Global tuberculosis control. WHO report.WHO/HTM/TB/2010.7 Geneva: World Health Organization, 2010.
8. Balasubramanian, R Fully intermittent six month regimens for pulmonary tuberculosis in South India. *Indian J Tuberc* 1991; **38(2)**: 51-3.
9. Jindani A, Nunn AJ, Enarson DA. Two 8-month regimens of chemotherapy for treatment of newly diagnosed pulmonary tuberculosis: international multicentre randomised trial. *Lancet* 2004; **364**: 1244–51.
10. Citron KM, Thomas GO. Ocular toxicity from ethambutol. *Thorax* 1986; **41**:737-9.
11. World Health Organization. Anti-tuberculosis drug resistance in the world, report no. 4.WHO/HTM/TB/ 2008.394 Geneva: World Health Organization Press; 2008.
12. Low rate of emergence of drug resistance in sputum positive patients treated with short course chemotherapy. *Int J Tuberc Lung Dis* 2001: **5(1)**: 40-45.
13. Korenromp EL, Scano F, Williams BG, Dye C, Nunn P. Effects of human immunodeficiency virus infection on recurrence of tuberculosis after rifampicin-based treatment: an analytical review. *Clin Infect Dis* 2003; **37(1)**: 101-2.
14. Mallory KF, Churchyard GJ, Kleinschmidt I, De Cock KM, Corbett EL. The impact of HIV infection on recurrence of tuberculosis in South African gold miners. *Int J Tuberc Lung Dis* 2000; **4(5)**: 455-62.
15. World Health Organization.Improving the diagnosis and treatment of smear-negative pulmonary and extrapulmonary tuberculosis among adults and adolescents: recommendations for HIV-prevalent and resource-constrained settings.WHO/ HTM/TB/2007.37. Geneva: World Health Organization, 2007.

Original Article

FNAC IN TUBERCULOUS LYMPHADENITIS: EXPERIENCE FROM A TERTIARY LEVEL REFERRAL CENTRE

Paliwal Nidhi[1], Thakur Sapna[1], Mullick Shalini[2] and Gupta Kumud[3]

(Received on 20.1.2011. Accepted after revision on 15.6.2011)

Summary

***Background*:** In developing countries like India, tuberculous lymphadenitis is one of the most common causes of lymphadenopathy. However, anti-tubercular treatment cannot be given only on clinical suspicion. Cytomorphology with acid fast staining proves to be a valuable tool in diagnosing these cases.

Aims: To study the utility, limitations of fine needle aspiration cytology and various cytomorphological presentations in reference to Ziehl-Neelsen staining in tuberculous lymphadenitis.

***Material and Methods*:** In a study period of July to October 2010, three hundred and eighteen consecutive superficial lymph nodes, clinically suspected to be tuberculous were subjected to cytological evaluation with Hematoxylin & Eosin, Giemsa and Ziehl-Neelsen stained smears. In addition, demographic profile of these patients with clinical presentation was also studied.

***Results*:** Incidence of tuberculous lymphadenitis was 55%. Overall AFB positivity was 71.0%. Only Necrosis without epithelioid cell granulomas was the most common cytological picture and that showed highest AFB positivity also. Three-fourth of the patients presented in second to fourth decade of life. Cervical region was the most common site of involvement with solitary lymphadenopathy as the most common presentation in contrast to matted lymph nodes as reported by others.

***Conclusions*:** Fine needle aspiration cytology is a safe, cheap procedure requiring minimal instrumentation and is highly sensitive to diagnose tuberculous lymphadenitis. The sensitivity can be further increased by complementing cytomorphology with acid fast staining. In acid fast staining negative cases, yield of acid fast bacilli positivity can be increased by doing Ziehl-Neelsen staining on second smear or decolourized smear revealing necrosis or by repeat aspiration. Microbiological assessment should also be done in such cases. **[*Indian J Tuberc 2011; 58:* 102-107]**

***Key words*:** Cytomorphological patterns, Tuberculous lymphadenitis, Ziehl-Neelsen staining

INTRODUCTION

Fine Needle Aspiration Cytology (FNAC) is almost safe, cost effective and conclusive procedure.[1] It provides an alternative to excision biopsy for lymph nodes and is an easy procedure for collection of material for cytomorphological and bacteriological examination.[2] Tuberculous lymphadenitis is a very common cause of superficial lymphadenopathy in countries like India. The aim of this study was to describe various cytological pictures of tuberculous lymphadenitis with their relative frequency and to assess correlation between FNAC and Ziehl-Neelsen (Z-N) staining in diagnosing tuberculous lymphadenitis.

MATERIAL AND METHODS

Three hundred and eighteen consecutive superficial lymph nodes, clinically suspected to be tuberculous, were aspirated for cytological evaluation after thorough clinical examination in a study period of July to October 2010. Aspirations were performed using 22 G needle and disposable 10 ml plastic syringe with a detachable syringe holder. In all the cases, alcohol fixed smears were made and stained with Hematoxylin & Eosin, one air-dried smear was stained with Giemsa stain, one smear was stained with Z-N technique (hot method) and an additional slide was kept unstained for any further required stain. The cytology smears revealing features of tuberculous

1. Senior Resident 2. Specialist 3. Sr. Pathologist

Department of Pathology, LRS Institute of TB & Respiratory Diseases, New Delhi.

Correspondence: Dr. Paliwal Nidhi, Senior Resident, Department of Pathology, LRS Institute of TB & Respiratory Diseases, Sri Aurobindo Marg, New Delhi – 110 030; Email: lrspathology@yahoo.in; Phone: 9278749894, 011-26854929.

lymphadenitis were grouped into four categories: epithelioid granulomas with caseous necrosis, epithelioid granulomas without necrosis, necrosis only without epithelioid granulomas and polymorphs with necrosis with or without epithelioid granulomas.[3] In addition, demographic profile of tuberculous patients with their present and past treatment history and clinical characteristics of lymphnodes were also studied.

RESULTS

Out of three hundred and eighteen superficial lymphnodes aspirated,125 cases showed AFB positivity (of which smears initially AFB negative showed positivity by doing Z-N staining on decolourized smears) while 51 cases were AFB negative with cytological picture of tuberculous lymphadenitis, 58 cases revealed reactive lymphnode hyperplasia and 84 cases included inadequate samples, lymphomas, metastases, etc. Among tuberculous cases, 77% of males and 75% of females were in the second to fourth decades of life with male to female ratio of 1:1.2 (Tables 1 and 2). Majority (50%) of the patients came to the institute from DOTS non-area (area in Delhi but not covered in DOTS area under our institute), 23% from DOTS area and 27% were from outside Delhi. Forty-eight patients had history of tuberculosis in the past and 42 patients were already on ATT at the time of aspiration. The cervical region was the most common site; involved in 90% cases, followed by axillary (6.4%) and inguinal (1.6%). Only three cases presented with generalized lymphadenopathy. In our study, most common presentation was single palpable cervical lymphnode in 63.3% of cases followed by multiple unilateral cervical lymphadenopathy in 19.2% of cases and multiple bilateral cervical lymphadenopathy in 7.2% of cases. Grossly purulent material was aspirated in 61.6%, caseous or cheesy material in 23.3% and blood mixed material in 15.1% of AFB positive cases while blood mixed material was the most common aspirate in 69.8% of AFB negative cases. Out of 176 cases showing cytological picture of tuberculous lymphadenitis, smears revealed epithelioid granulomas with caseous necrosis in 16.4% of cases , epithelioid granulomas without necrosis in 14.3% of cases, necrosis only without epithelioid granulomas

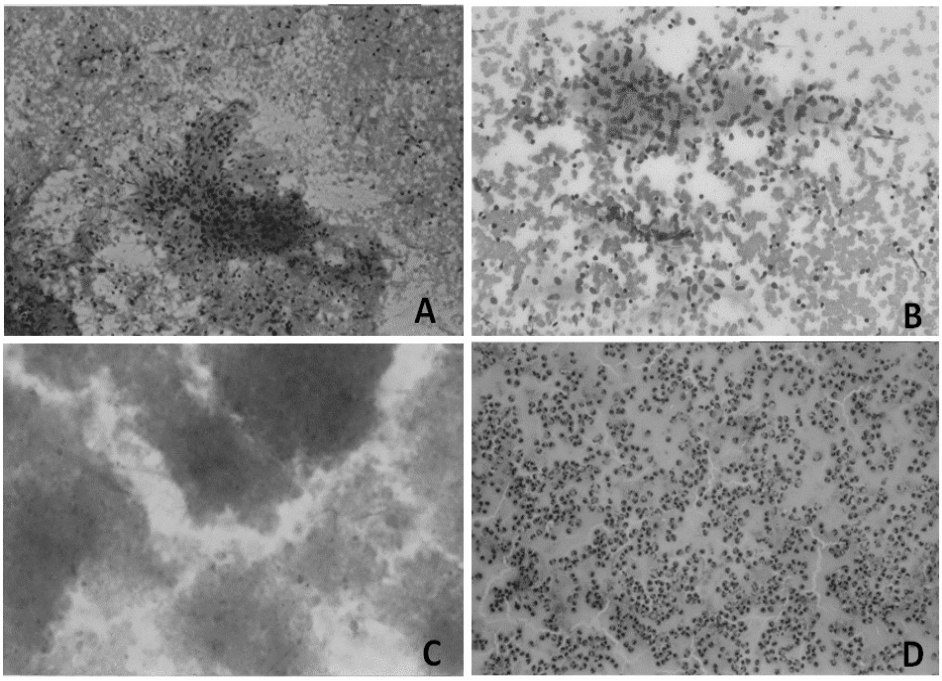
Table 1: Incidence of reactive *versus* tuberculous lymphadenopathy in male and female

Diagnosis	Male	Female	Total
Reactive lymph node hyperplasia	38	20	58
Tuberculous lymphadenopathy	81	95	176
Total	119	115	234

Table 2: Incidence of tuberculous lymphadenopathy in relation to age and sex

Age group	Male	Female	Total	%
1-10 yrs	5	9	14	7.9
11-20 yrs	16	18	34	19.4
21-30 yrs	25	32	57	32.3
31-40 yrs	22	21	43	24.5
41-50 yrs	9	9	18	10.3
50 yrs and above	4	6	10	5.6
Total	81	95	176	100

in 39.2% of cases and polymorphs with necrosis with or without epithelioid granulomas in 30.1% of cases (Figs.1.A,B,C,D). AFB positivity was found in 69.5% of the cases showing epithelioid granulomas with caseous necrosis, 3.2% of cases with epithelioid granulomas without necrosis, 85.5% of cases with necrosis only without epithelioid granulomas and 79.2% of cases with polymorphs with necrosis with or without epithelioid granulomas (Table 3). Overall AFB positivity was seen in 71.0% cases.



Various cyto-morphological patterns in tuberculous lymphadenitis

Fig. 1.A: Epithelioid cell granuloma with necrosis. (H&E 100X)
Fig. 1.B: Epithelioid cell granuloma without necrosis. (H&E 100X)
Fig. 1.C: Only necrosis, no granulomas. (H&E 100X)
Fig. 1.D: Only neutrophils, no granulomas. (H&E 100X)

Table 3: Various cytomorphological pictures in tuberculous lymphadenopathy

Cytomorphological picture	No. of cases	%	AFB positive cases	AFB negative cases
Epithelioid granulomas with caseous necrosis	29	16.4	20(69.5%)	9(30.5%)
Epithelioid granulomas without necrosis	25	14.3	4(3.2%)	21(96.8%)
Necrosis only without epithelioid granulomas	69	39.2	59(85.5%)	10(14.5%)
Polymorphs with necrosis	53	30.1	42(79.2%)	11(20.8%)
Total	176	100	125	51

DISCUSSION

Superficial lymphadenopathy is a very common clinical finding, aetiology of which can be suspected by clinical signs and symptoms. However, a morphological diagnosis is essential to start anti-tuberculous treatment in cases of tuberculous lymphadenopathy. FNAC lymph node is a simple, non-invasive, cheap tool with high sensitivity in tuberculous cases and can replace excision biopsy for diagnosing tuberculosis in developing countries like India. Tuberculous lymphadenopathy can be seen in patients ranging from early to advanced age. In our study, the youngest patient was four-year-old and the oldest was 63 years' old. In a study by Ahmad *et al*, the youngest patient was two-year-old and the oldest being 95 years.[4] Majority of the patients (75%) were in the second to fourth decades of life. Similar age distribution was seen in a study by Ergete and Bekele[2], Purohit *et al*[5] and Dandapat *et al*[6]. A slight female predominance with 1:1.2 sex ratio was seen in our study. Similarly, female predominance was noted by Pamra *et al*[7], Ergete and Bekele[2] and Purohit *et al*[5] while male predominance was noted by Rajsekaran *et a l*[8], and Ahmad *et al*.[4] Clinically, in our study, cervical region was the most commonly affected region, involved in 90% of cases. This was in concordance with Bezabih *et al*[9] who observed cervical involvement in 74.2% of cases. A study conducted by Sharma *et al*[10] in pediatric age group also showed similar results with female predominance and most common involvement of cervical region (88.2%). While matted lymph nodes were seen in majority of cases (60%) by Ahmad *et al*[4], in our study 63.3% of cases presented with solitary lymphadenopathy. Single lymph node enlargement was seen in 48.6% cases of tubercular lymphadenopathy by Aggarwal *et al*.[11] We noted a much higher incidence(55%) of tuberculous lymphadenopathy while Ahmad *et al*[4] found 38% and Tilak *et al*[12] 38.8% cases of tuberculous lymphadenopathy. The high incidence noted by us may be because our institute is a referral centre for tuberculosis cases.

Most common cytological pattern seen was necrosis only without granulomas in 39.2% of cases and polymorphs with necrosis in 30.1% of cases. While in a study by Gupta *et al*, epithelioid clusters with or without Langhan's giant cells with necrosis was most commonly observed cytological pattern in 50.35 cases.[13] This is also the classic pattern, commonly seen in excision specimens of tuberculous lymphnodes (Fig. 2). Highest AFB positivity was seen in smears revealing necrosis only without epithelioid granulomas (85.5%) and polymorphs with necrosis

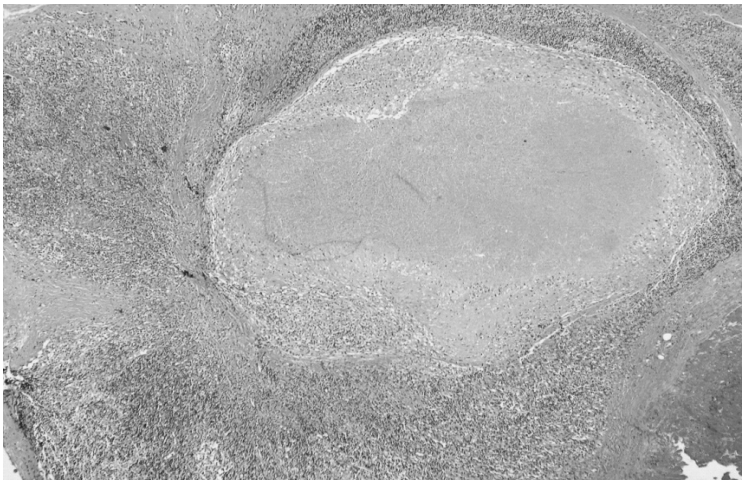


Fig. 2: Histological picture of tuberculous lymphadenitis showing necrotizing granulomatous lymphadenitis (H&E 40X)

with or without epithelioid granulomas (79.2%) while the lowest was seen in smears showing epithelioid granulomas without necrosis(3.2%). Bezabih *et al*[9] found the highest AFB positivity in cases showing necrosis only without epithelioid granulomas (69.7%) and the lowest in cases showing epithelioid granulomas without necrosis (20.0%). Similarly, the highest AFB positivity (75.6%) was seen in smears revealing necrosis only without epithelioid granulomas by Gupta *et al*.[13] Maximum AFB positivity (61.6%) was found in smears containing purulent material on aspiration. Similarly Ahmad *et al*[4] noted 68.8% AFB positivity in smears containing purulent material on aspiration. In our study, overall AFB positivity was seen in 71% of cases. AFB positivity was observed in 71.7% of cases by Ergete and Bekele[2], 59.4% cases by Bezabih *et al*[9], 45.6% cases by Dasgupta *et al*[14] and 19.6% cases by Aggarwal *et al*.[11] High AFB positivity noted in our study may be because of extensive screening done as in addition to one Z-N stained smear in each case, we got Z-N staining done on second smear or decolourized smear where cytology suggested tuberculosis, specially when necrosis was present. Yield of AFB positivity can further be increased by doing repeat FNAC of lymphnode.[15] AFB negative cases revealing only epithelioid cell granulomas without necrosis should be clinically correlated with microbiological assessment. Similarly, atypical cells should be ruled out in smears showing necrosis only without epithelioid cell granulomas and AFB negativity and material should be submitted for culture. Microbiological assessment is necessary in AFB negative cases to confirm the diagnosis of tuberculosis as approximately 10,000- 100,000 mycobacterial organism/ml of sample should be present for smear AFB positivity.

CONCLUSION

FNAC can be performed as outpatient department procedure in superficial lymphadenopathy cases. Procedure is safe, well accepted by patients, very cost-effective and requires minimum instrumentation in comparision to excision biopsy. Diagnostic accuracy as high as 100% in tuberculous lymphadenopathy cases has been reported by Tripathy *et al*[16], 84.4% by Dasgupta *et al*[14], 83.3% by Dandapat *et al*[6] and 87% by Narang.[17] Therefore even in most remote areas, FNAC can be used for diagnosing tuberculous lymphadenopathy. Coupling FNAC with Z-N staining increases the diagnostic accuracy. Diagnostic accuracy can be further increased by submitting some material obtained by FNA for culture.

REFERENCES

1. Chaturvedi NK, Singh JP, Das Amita. Fine needle aspiration cytology in the diagnosis of tuberculous lymphadenitis. *Indian J Pathol Microbial* 1989; **32(2)**: 101-4.
2. Ergete W and Bekele A. Acid fast bacilli in aspiration smears from tuberculous patients. *Ethiop J Health Dev* 2000; **14(1)**: 99-104.
3. Heerde PV, Miliauskas J, Field A. Lymphnodes. In: Orell SR, Sterrett GF, Whitaker D,ed Fine needle aspiration cytology; 4th ed; New York: Churchill Livingstone 2005: pp 83-124.
4. Ahmad SS, Akhtar S, Akhtar K, Naseem S, Mansoor T, Khalil S. Incidence of tuberculosis from study of fine needle aspiration cytology in lymphadenopathy and acid fast staining. *Ind J Community Medicine* 2005; **30(2)**: 63-5.
5. Purohit MR, Mustafa T, Morkve O, Sviland L. Gender differences in the clinical diagnosis of tuberculous lymphadenitis - a hospital-based study from central India. *International Journal of Infectious Diseases* 2009 Sep; **13(5)**: 600-05.
6. Dandapat MC, Panda BK, Patra AK, Acharya N. Diagnosis of tubercular lymphadenitis by fine needle aspiration cytology. *Indian J Tuberc* 1987; **37**: 139-142.
7. Pamra S, Baily GVS, Gupta SP *et al*. Cervical lymphadenopathies. *Indian J Tuberc* 1987; 96-100.
8. Rajsekaran S, Gunasekaran M, Bhanumati V. Tuberculous cervical lymphadenitis in HIV positive and negative patients. *Indian J Tuberc* 2001; **48**: 201-4.
9. Bezabih M, Mariam DW, Selassie SG. Fine needle aspiration cytology of suspected tuberculous lymphadenitis. *Cytopathology* 2002; **13(5)**: 284-90.
10. Sharma S, Sarin R, Khalid UK, Singla N,Sharma PP, Behera D. Clinical profile and treatment outcome of tuberculous lymphadenitis in children using DOTS strategy. *Indian J Tuberc* 2010; **57**: 4-11.
11. Aggarwal P, Wali JP, Singh S, Handa R, Wig N, Biswas A. A clinico-bacteriological study of peripheral tuberculous lymphadenitis. *J Assoc Physicians India* 2001 Aug; **49**: 808-12.
12. Tilak V, Dhadel AV, Jain R. Fine needle aspiration cytology of head and neck masses. *Ind J Pathol Microbiol* 2002; **45(1)**: 23-30.
13. Gupta AK, Nayar M, Chandra M. Critical appraisal of fine needle aspiration cytology in tuberculous lymphadenitis. *Acta Cytol* 1992 May-Jun; **36(3)**: 391-4.

14. Dasgupta A, Ghosh RN, Poddar AK *et al*. Fine needle aspiration cytology of cervical lymphadenopathy with special reference to tuberculosis. *J Indian Med Assoc* 1994; **92(2)**: 44-6.
15. Kumar N, Jain S, Murthy NS. Utility of fine needle aspiration in acute suppurative lesions. Follow-up of 263 cases. *Acta Cytol* 2004 May-Jun; **48(3)**: 337-40.
16. Tripathy SN, Mishra N, Patel MM, Samantray DK, Das BK, Mania RN. Place of aspiration biopsy in the diagnosis of lymphadenopathy. *Indian J Tuberc* 1985; **32**: 130-4.
17. Narang RK, Pradhan S, Singh RP, Chaturvedi S. Place of fine needle aspiration cytology in the diagnosis of lymphadenopathy. *Indian J Tuberc* 1990; **37**: 29.

IUATLD WORLD CONFERENCE

The 42nd Union World Conference on Lung Health will be held in Lille (France) from 26th to 30th October, 2011. The theme of the conference is "Partnerships for scaling-up and care".

For more details, please visit: Lille2011@theunion.org

Original Article

CORRELATION OF SPUTUM SMEAR STATUS WITH CD4 COUNT IN CASES OF PULMONARY TUBERCULOSIS AND HIV CO-INFECTED PATIENTS - A HOSPITAL BASED STUDY IN A RURAL AREA OF CENTRAL INDIA

Sameer Singhal[1], S. N. Mahajan[2], S. K. Diwan[3], Abhay Gaidhane[4] and Z. S. Quazi[4]

(Received on 24.10.2010. Accepted after revision on 13.5.2011)

Summary

***Background*:** In HIV-infected patients, PTB (Pulmonary Tuberculosis) is still the commonest form of TB. The most cost-effective method of detecting TB cases among PTB suspects in high-prevalence countries is by sputum smear microscopy. World Health Organisation (WHO) states that sputum positivity decreases accompanying with atypical chest x-ray findings as CD4 count decreases. This expectation that infection with HIV would reduce the sensitivity of acid-fast smears, due to a decreased frequency of cavitary pulmonary MTB, has not been substantiated in a few studies done in the past. This study was undertaken to see the correlation of sputum smear status with CD4 count in cases of Pulmonary Tuberculosis HIV co-infected patients in our institute, being a tertiary referral centre.

***Methodology*:** In our hospital based cross-sectional study, 98 patients having PTB-HIV co-infection were followed and acid fast smear positivity status was assessed in correlation with chest radiograph and CD4 count.

***Results*:** Acid-fast smear positivity to negativity was almost 1:1 in CD4 count between 0-200 whereas it was 3:1 in cases of CD4 count above 200. There was significant difference (p value 0.013) in two groups with CD4 count cut-off value 200 which endorses the fact by WHO that sputum smear negativity increases with increase in degree of immunosupression.

***Conclusion*:** Sputum examination remains an important diagnostic tool for pulmonary tuberculosis in immunocompromised host with CD4 count above 200 but there is an urgent need for better diagnostic methods in CD4 count below 200. **[*Indian J Tuberc 2011; 58:* 108-112]**

***Key words*:** PTB-HIV Co-infection, WHO, CD4 count

INTRODUCTION

As Human immunodeficiency virus (HIV) infection progresses, CD4+ T-lymphocytes decline in number and function. These cells play an important role in the body's defence against tubercle bacilli. Thus, the immune system becomes less able to prevent the growth and local spread of *M. tuberculosis*[1,2]. In HIV-infected patients, PTB is still the commonest form of tuberculosis (TB). The most cost-effective method of detecting TB cases among PTB suspects in high-prevalence countries is by sputum smear microscopy. Sometimes, a patient may be negative on sputum smear microscopy but may not improve on a broad-spectrum antibiotic. According to National guidelines, if clinician still suspects TB, reassess the patient and do a chest radiograph (CXR). If the CXR is typical of PTB, register the patient with the District Tuberculosis Officer (DTO) and start TB treatment. If doubtful about the CXR diagnosis of TB, e.g. if the CXR shows non-specific pulmonary infiltrates, give the patient another course of antibiotics. If there is no clinical improvement, or if the cough disappears only to return shortly afterwards, repeat sputum smear microscopy. If clinician still thinks that the patient may have TB despite, further negative sputum smears, again reassess the patient and repeat the CXR. Then decide whether the diagnosis is TB or not. In cases, where diagnostic doubt persists, sputum culture may be useful if suitable facilities are available[3]. The chest radiograph presentation depends on the degree of immunosuppression. 'Early HIV' often resembles post-primary TB with sputum smear positive and cavities on chest radiograph in contrast to 'Late HIV' which resembles primary TB with sputum smear mostly negative and disseminated infiltrations on chest radiograph but no cavities. This study was undertaken to see the correlation of sputum smear status with CD4

1. Associate Professor and HOD* 2. Professor and HOD** 3. Professor** 4. Associate Professor***
Departments of Pulmonary Medicine*, Medicine** and Community Medicine***, AVBRH, JNMC, DMIMS (DU), Wardha, (Maharashtra)
Correspondence: Dr Sameer Singhal, Associate Prof and HOD, Department of Pulmonary Medicine, AVBRH, JNMC, DMIMS (DU), Wardha, (Maharashtra).
Email: singhal_sameer@yahoo.co.in; Phone: 09970841052.

count in cases of Pulmonary Tuberculosis HIV co-infected patients in our institute, being a tertiary referral centre.

MATERIAL AND METHODS

This was a hospital-based cross-section observational study.

Subjects

All cases diagnosed as Pulmonary Tuberculosis as per diagnostic algorithm given by WHO and who had HIV co-infection were included in this study.

Data Collection and Lab procedure

At the baseline, all potential subjects had a physical examination and standardized interview that included questions about weight loss in the past three months and about the presence and duration of any cough or fever. All subjects had a baseline chest x-ray and submitted three expectorated sputum samples for microbiologic testing. Subjects were instructed to the need for deep cough and asked to provide one spot sputum sample and to label and bring two first morning samples; subjects who were unable to produce a spot specimen were asked to bring three first morning samples. Sputum samples were decontaminated using the modified Petroff's method and concentrated by centrifugation at 3000 g for 15 minutes. Smears were screened by auramine staining and positive smears were counterstained by the Ziehl Neelsen (ZN) staining technique without removing the auramine. Smears were read without knowledge of culture outcomes and results were categorized as 3+ (> 10 AFB/oil field), 2+ (1 – 10 AFB/oil field), 1+ (10 – 99 AFB/100 oil fields), scanty (1 – 9 AFB/ 100 oil fields) and negative (0 AFB/100 oil fields). For each smear, a total of 100 microscopic fields were examined as per protocol. Each sample was then cultured in both pyruvate and glycerol containing Lowenstein Jensen media at 37°C for up to eight weeks. Plates were examined weekly for growth. Colonies were identified according to criteria based on the speed of growth and macroscopic features e.g. roughness and pigment production. Culture results were expressed as actual number of colonies (if less than 20 colonies/slant) 1+ (20–100 colonies/slant, 2+ (discrete innumerable colonies/ slant) and 3+ (for confluent growth). Quality assurance was accomplished by assessing the quality and adequacy of specimens, and by monitoring microscopy and culture procedures, preparation and storage of reagents and performance of equipment against established laboratory operating procedures. Patients were requested to provide an additional specimen in case of submitting either an inadequate or salivary sample. For smear microscopy, positive and negative control slides were included with each batch of new reagents and, in a blind manner, when reading patient smears.

Measured Parameters

Following data were recorded; three sputum smears for AFB examination, CD4 count at the time of diagnosis and chest radiograph findings at the time of diagnosis. All slides were read independently by three experienced microscopists, and kept for up to three months for external quality control. Review of smear and culture results provided an internal quality assurance measure. Additional quality measures for cultures included monitoring of; quality of water, decontamination, digestion, and concentration procedures, inspissation and incubation temperatures, and measurement and adjustment of pH of culture media. A standard laboratory strain *M. tuberculosis* H37Rv was used as a positive control. Human immunodeficiency virus testing was done after pretest counselling and written informed consent. The diagnosis of HIV infection was based on three positive tests (Tridot, J. Mitra and Comb ADIS, Span Diagnostics) followed by an ELISA (Lab System, U.K.). A posteroanterior chest radiograph was done. The diagnosis of pulmonary tuberculosis was based on sputum smear and culture results along with clinical and radiographic features. The CD4 count was done for all HIV positive patients by flow cytometry. Patients with long term steroid therapy, diabetes, and other causes of immunosuppresion or having MOTT infection were excluded from the study.

Treatment and Follow up

Patients diagnosed to have pulmonary tuberculosis were treated with DOTS treatment under

RNTCP with two months of ethambutol (1200 mg), INH (600 mg), rifampicin (450/600 mg) based on body weight < 60 kg: 450 mg and > 60 kg: 600 mg] pyrazinamide (1500 mg) given three times a week followed by four months of INH (600 mg) and rifampicin (450/600mg) given thrice weekly. Treatment was supervised completely in the initial intensive phase and once a week in the continuation phase. The patients were followed up every month with a clinical examination and three sputum examinations. A chest radiograph was repeated at the end of treatment. An independent assessor (NMS) who did not know the clinical background of the patient including HIV status, CD4 and sputum smear status read all the chest radiographs.

HAART treatment was started in all PTB-HIV co-infected patients, preferably within first eight weeks of starting DOTS.

Statistical Analysis

Data was entered into excel and analysis was done using SPSS software version 13. All tests were evaluated at a significance level of 0.05. Fisher Exact Probability Test was calculated. The research protocol was approved by the Ethics Committee of the Datta Meghe Institute of Medical Sciences.

RESULTS

Total of 2000 patients of tuberculosis were followed during year Jan 2007- Jan 2010. Out of these, 850 (42.50%) were diagnosed to have pulmonary tuberculosis. Out of these 850 patients, 98 (11.52%) were having PTB-HIV co-infection. Acid fast smear positivity status was assessed in correlation with chest radiograph (Table 1) and CD4 count (Table 2). Treatment outcome was seen in different subgroups of CD4 counts (Table 3).

Table 1: Chest radiograph appearance in Sputum Smear Positive *versus* Sputum Smear Negative patients

Chest Radiograph findings	Total Number	Sputum AFB Positive	Sputum AFB Negative
Cavity	2	1	1
Normal	5	4	1
LZ Pneumonia	13	9	4
Disseminated (Bronchogenic Spread)	78	40	38

Table 2: CD4 count (Degree of Immunosupression) in correlation to sputum smear status

CD4 Count	Sputum for Acid Fast Bacilli (AFB)		Total
	Negative	Positive	
	No (%)	No (%)	
Less or equal to 200	40 (41%)	39 (40%)	79 (81%)
More than 200	4 (4%)	15 (15%)	19 (19%)
Total	44 (45%)	54 (55%)	98 (100%)

* Applying Fisher Exact Probability Test p value is 0.013 (Significant) suggesting that there was a significant difference between two groups.

Table 3: CD4 count in correlation with treatment outcome

CD4 count	Cured/Treatment Completed	Death	Defaulter	MDR TB	Loss to Follow Up
0-200	23	10	1	1	44
201-400	1	1	1	-	12
>400	1	1	-	-	2

DISCUSSION

Around 2.5 million people are infected with HIV in India. Estimated 40% of the Indian population is infected with *M. tuberculosis*. Estimated one million persons are co-infected with *M. tuberculosis* and HIV. Risk of developing TB is higher in HIV infected persons. Life-time risk of developing TB is 60% in persons infected with both HIV and TB. HIV infected person develops the disease rapidly as compared to HIV negative. The rate of progression to disease is 10-30 times higher in HIV infected persons. On the other hand, HIV increases the risk of developing other opportunistic infections. TB is a common cause of death in AIDS patients. Active TB disease is the commonest opportunistic infection amongst HIV-infected individuals and is also the leading cause of death in PLHA (People living with HIV/AIDS). Surveys in India show 1% to13% HIV amongst TB patients[3]. Even in HIV-infected patients, pulmonary TB is still the commonest form of TB[4]. HIV-infected, smear positive patients tend to excrete significantly fewer organisms per ml of sputum than HIV-negative patients[5], which can lead to AFB being missed if the appropriate number of sputum samples as well as high power fields is not examined by microscopy. Pitchenik[6] expressed concern that immunosuppression resulting from human immunodeficiency virus (HIV) type 1 may not only reduce the sensitivity of the sputum smear by reducing caseation necrosis, and thus the number of acid-fast bacilli in the airway, but may also have affected the specificity of the sputum smear by increasing the proportion of patients with non-tuberculous mycobacteria. They found that sputum smear was significantly (P<.05) less likely to be positive for acid fast bacilli in HIV seropositive (50/74, 68%) than in HIV seronegative (172/215, 80%) patients. Likewise, a sputum culture positive for *M. tuberculosis* was less likely (P=.05) to be present in HIV seropositive (61/74, 82%) compared to HIV seronegative patients (196/215, 91%)[7]. Chest X-rays play a significant role in shortening delays in diagnosis and should be performed early in the course of investigation of a tuberculosis suspect. WHO states that sputum positivity decreases in an HIV infected patient accompanying with atypical chest x-ray findings as CD4 count decreases. This expectation that infection with HIV would reduce the sensitivity of acid-fast smears, due to a decreased frequency of cavitary pulmonary MTB, has not been substantiated in a few studies done in the past[8,9]. Smith *et al*[8] in a study showed that positive acid-fast sputum smears in culture-proven MTB occur with similar frequency in patients with and without HIV. The absence of cavitary disease did not significantly reduce the frequency of positive acid-fast smears. For patients with HIV, the likelihood of a positive smear was also independent of CD4 cell counts and drug resistance. Patients with HIV and disseminated MTB had positive sputum smears in nearly all cases. The sputum negativity tends to increase as the HIV disease and immune suppression progress. Klein *et al*[10] showed a decreased sensitivity of sputum smears in culture-positive MTB among patients with HIV infection (45 per cent vs 81 per cent among patients without HIV). Long *et al*[7] observed a 66 per cent frequency of positive acid-fast sputum smears in HIV-infected patients, compared with 78 per cent in patients without HIV infection. Modilevsky *et al*[11] found an 83 per cent frequency of positive acid-fast smears in PTB-HIV co-infected patients in comparison to 16 per cent in pulmonary MAC-HIV co-infected patients.

They concluded that more intensive diagnostic use of sputum acid-fast smears may improve the outcome in patients with tuberculosis. Pitchenick *et al* and Theuer *et al*[12] observed no differences in the frequency of positive acid-fast smears between HIV-infected and non-HIV-infected patients. In our study, acid-fast smear positivity to negativity was almost 1:1 in CD4 count between 0-200 whereas it was 3:1 in cases of CD4 count above 200, which suggests that sputum positivity decreases as CD4 count decreases; but have almost equal proportion with sputum negativity in CD4 counts below 200, which is the case similar to general population. Also, there was significant difference (p value 0.013 one tailed and 0.020 two tailed) in two groups with CD4 count cut-off value 200 which endorses the fact by WHO that sputum smear negativity increases with increase in degree of immunosupression. Maximum numbers of tubercular cases were seen in CD4 count between 0-200 which correlates with the fact that tuberculosis is most common opportunistic infection in HIV case with CD4 count below 250. Proportion of typical chest x-ray findings in cases with CD4 count between 0-200 was only 3.6%.

Limitation of the Study

Survival rates were not assessed in cases with early initiation of Highly Active Antiretroviral Therapy (HAART) in CD4 count between 0-200. Follow up treatment could be assessed in only 40.98% cases as most of cases were of out of district.

CONCLUSION

This study endorses the fact that there is more sputum smear negativity in patients with CD4 count below 200 as stated by WHO. Sputum examination remains an important diagnostic tool for pulmonary tuberculosis in immunocompromised host with CD4 counts above 200 but there is an urgent need for better diagnostic methods in patients with CD4 counts below 200 in view of high sputum smear negativity.

REFERENCES

1. Dannenberg AM. Immune mechanisms in the pathogenesis of pulmonary tuberculosis. *Rev Infect Dis* 1989; **11**: S369-S78.
2. Nunn PP, McAdam KPWJ. Mycobacterial infections and AIDS. *Br Med Bull* 1988; **44**: 801-13.
3. Swaminathan S and Narendran G. HIV and tuberculosis in India. *J. Biosci* 2008; **33**: 527–37.
4. World Health Organization. TB/HIV A Clinical Manual. Geneva. (WHO/HTM/TB/2004.329)
5. American Thoracic Society. Diagnostic standards and classification of tuberculosis in adults and children. *Am J Respir Crit Care Med* 2000; **161**: 1376-95.
6. Pitchenik AE, Rubinson HA. The radiographic appearance of tuberculosis in patients with the Acquired Immune Deficiency Syndrome (AIDS) and pre-AIDS. *Am Rev Respir Dis* 1985; **131(3):** 393-6.
7. Long R, Scalcini M, Manfreda L, Baptiste MJ, and Hershfield E. The impact of HIV on the usefulness of sputum smears for the diagnosis of Tuberculosis. *Am J Public Health* 1991; **81**: 1326-8.
8. Smith RL, Yew K, Berkowitz KA and Aranda CP. Factors affecting the yield of acid-fast sputum smears in patients with HIV and Tuberculosis. *Chest* 1994; **106**: 684-6.
9. Chaisson RE, Schecter GF, Theuer CP, Rutherford GW, Echenberg DF, Hopewell PC. Tuberculosis in patients with the acquired immunodeficiency syndrome. Clinical features, response to therapy, and survival. *Am Rev Respir Dis* 1987; **136(3):** 570-4.
10. N C Klein, F P Duncanson, T H Lenox, 3rd, A Pitta, S C Cohen and G P Wormser. Use of mycobacterial smears in the diagnosis of pulmonary tuberculosis in AIDS/ARC patients. *Chest* 1989; **95**: 1190-2.
11. Modilevsky T, Sattler FR, Barnes PF. Mycobacterial disease in patients with human immunodeficiency virus infection. *Arch Intern Med* 1989; **149**: 2201-05.
12. Theuer CP, Hopewell PC, Elias D, Schecter GF, Rutherford GW, Chaisson RE. Human immunodeficiency virus infection in tuberculosis patients. *J Infect Dis* 1990; **162**: 8-12.

Original Article

PROFICIENCY PANEL TESTING - A RELIABLE TOOL IN EXTERNAL QUALITY ASSESSMENT OF SPUTUM SMEAR MICROSCOPY SERVICES IN GUJARAT, INDIA

Paresh V. Dave*, Nilesh D. Patel†, K. Rade‡, Rajesh N. Solanki†, P. G. Patel*, P. Patel*, N. Joshi*, B. Modi† and K. Pujara*

(Received on 31.1.2011; Accepted after revision on 13.5.2011)

Summary

***Objective*:** To assess the proficiency of Senior TB Laboratory Supervisors (STLSs) and district level Laboratory Technicians (LTs) in sputum smear microscopy.

Method: Intermediate Reference Laboratory (IRL), Ahmedabad had manufactured and validated Proficiency Panel Testing slides from sputum samples, made On Site Evaluation (OSE) visits of District TB Centres (DTCs) in two rounds, and conducted Proficiency Panel Testing of STLSs & DTC-LTs from January 2005 to June 2009.

***Result*s:** High level of concordance in Z-N smear grading was found between Microbiologist and district laboratory staff. DTC readers reported overall consistency level of more than 98% in Z-N grade agreement during both the IRL, EQA, OSE visits. The tendency to over-grade the panel slides was much higher (more than 22%) as compared to under-grade (less than 2%) them in "correct slides". High False Positive (HFP) error was not observed in the present study.

Conclusion*:** Laboratory supervisor's proficiency can be quickly assessed by Proficiency Panel Testing, under multi-level quality assurance network system of sputum smear microscopy in public health programmes like the RNTCP. Proficiency Panel Testing is highly replicable and reproducible tool for quick and reliable assessment of proficiency of the staff and it can be made more effective by raising the proportion of lower grade positive slides in panel set of each reader. DTC readers' overall agreement level of more than 98% in Z-N grade suggests high level of precision and excellent consistency during both the IRL, EQA, OSE rounds. It is concluded that even for a large network of sputum smear microscopy centres under public health programmes like the RNTCP in order to take corrective action, Proficiency Panel Testing can be effectively used for quick identification of suboptimal- technical performance of the supervisory staff. ***[Indian J Tuberc 2011; 58: 113-119]

***Key words*:** Proficiency Panel Testing, Sputum smear microscopy, IRL, EQA, OSE, RNTCP

INTRODUCTION

In the DOTS strategy advocated by the World Health Organization (WHO), diagnosis of tuberculosis (TB) is mainly based on results of sputum smear microscopy. Because correct reading of sputum smears is critical to case finding and management, the DOTS strategy also recommends that quality control of smear microscopy be an integral part of national TB control programmes[1]. The establishment of a broad network of well-functioning peripheral laboratories within the context of the health system and readily accessible to the population is a high priority for any tuberculosis control programme. If the laboratory diagnosis is unreliable, all other activities will be affected[2]. In effective quality assurance (QA) system of the Revised National Tuberculosis Control Programme (RNTCP), sputum smear microscopy network is of crucial importance for the future of the programme. Therefore, quality assurance of laboratory services, including AFB sputum smear microscopy, is essential.

Quality Assurance (QA) is a system designed to continuously improve the reliability and efficiency of laboratory services. As defined by both the WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD), a quality assurance programme for AFB smear microscopy has several components:

- **Quality Control (QC):** A systematic internal monitoring of working practices, technical procedures, equipment, and material, including quality of stains.

*Department of Health & Family Welfare, State Government of Gujarat,

†Department of Medical Education, State Government of Gujarat,

‡World Health Organization, India Office, RNTCP-Technical Assistance Project

***Correspondence*:**Dr. Nilesh D. Patel; 7, Shreeji Apt., Near Swaminarayan Temple, Thakkarbapanagar approach, Bapunagar, Ahmedabad, Gujarat. Pin code 382350; Mob: 09909156635. Email ID: ndp1@rediffmail.com

• **External Quality Assessment (EQA):** A process to assess laboratory performance. EQA includes on-site evaluation of the laboratory to review QC and should include on-site re-reading of smears. EQA also allows participant laboratories to assess their capabilities by comparing their results with those obtained in other laboratories in the network (intermediate and central laboratory) through panel testing and rechecking.

• **Quality Improvement (QI):** A process by which the components of smear microscopy diagnostic services are analyzed with the aim of looking for ways to permanently remove obstacles to success. Data collection, data analysis, and creative problem solving are the key components of this process. It involves continued monitoring, identifying defects, followed by remedial action including retraining when needed, to prevent recurrence of problems. QI often relies on effective on-site evaluation visits[2]. EQA is also termed "proficiency testing" as described by IUATLD[3].

Quality in the network of designated sputum smear microscopy laboratories under the RNTCP is ensured through a hierarchical network of activities at various levels in the health system. Senior Tuberculosis Laboratory Supervisors (STLSs) for all designated microscopy centres in the Tuberculosis Unit (TU) ensure the implementation of Quality Assurance protocol[3,4].

Intermediate Reference Laboratory (IRL) at State Tuberculosis Training and Demonstration Centre (STDC), Ahmedabad has the overall responsibility of quality assurance in all 700 DMCs in the state of Gujarat and Union Territories (UTs) of Daman, Diu and Dadra Nagar Haveli. This is done by various activities including IRL On Site Evaluation (OSE) conducted as a part of External Quality Assessment (EQA) visits to District TB Centres (DTCs)[3,4].

Proficiency Panel Testing of the STLSs and LTs at DTCs during these visits is conducted using the standard panel slides manufactured and validated by the Microbiologist at IRL. The panel testing of the STLSs & DTC-LTs is an important tool for quick assessment of their proficiency in sputum smear microscopy.

MATERIAL AND METHODS

The data were collected and stripped off from IRL, EQA, OSE visit reports. Any individual patient/ person was not interviewed and a data analysis was part of duty of the programme officers and hence ethical committee clearance was not required.

Setting

This study was a part of the implementation of the RNTCP in the state of Gujarat and UTs. The staff of IRL, STDC, Ahmedabad, STLSs and DTC-LTs in the districts of Gujarat and UTs participated in the study which was conducted during the two rounds of IRL, EQA, OSE visits to all districts from January 2005 to June 2009.

Manufacturing of Proficiency Panel Testing slides

The panel slides were manufactured and validated at IRL, Ahmedabad as per External Quality Assessment guidelines of the Revised National Tuberculosis Control Programme in India. In brief, fresh, good quality Acid Fast Bacilli (AFB) negative sputum samples were pooled to yield 25-30 ml of formalinised "negative stock" sample. Minimum 5-6 ml 3+ grade (more than 50 AFB/ oil immersion field) muco-purulent sputum sample was formalinised as "positive stock" sample. Respective sputa were mixed with 2% N-Acetyl L-Cysteine (NALC)– 2.9% citrate solution, incubated in a shaker incubator for ten-fifteen minutes to liquefy and homogenize them. Negative stock was used to manufacture AFB negative smears and as a diluent for preparation of AFB positive smears from positive stock. All the sputum and smear preparations were carried out in Class II biological safety cabinets. The slides were manufactured using 4 mm diameter wire loop. All the slides were heat fixed by flaming method. Six slides from each grade were randomly selected, stained, read independently by minimum four IRL readers and results were tabulated for validation process. The manufactured slides had some known number of AFB per slide. The slides of different grades were kept in separate, designated

wooden boxes and stored in shelves for up to six months, for usage in Proficiency Panel Testing. This procedure was repeated for adequate number of slides for IRL, EQA, OSE visits. Only manufactured and validated panel slides were used throughout the study period in order to standardize test conditions and draw scientific data[3].

Proficiency Panel Testing during IRL EQA OSE visits

STLSs and DTC-LTs were given a panel slide set of five heat fixed unstained sputum slides per reader. The panel set essentially consisted of minimum one AFB negative, one AFB positive, and three slides of any grade. The panel slides were handed over to the reader along with standard recording and reporting forms. Each reader independently stained the slides by Ziehl-Neelsen (Z-N) stain, examined them, and reported results as per the RNTCP grading for sputum smear given in Table 1[5].

These activities took place under direct supervision of IRL personnel in order to ascertain time limit for each activity, to give on-site feedback, and to facilitate rechecking of discordant results, if any. Discrepant result was resolved by IRL Microbiologist as per EQA guidelines. Smear results were analysed for consistency as per Table 2[3,5].

Table 1: Grading of sputum smear on microscopic examination[5]

If the slide has:	Result	Grading	No. of fields to be examined
More than 10 AFB per oil immersion field	POS	3+	20
1-10 AFB per oil immersion field	POS	2+	50
10-99 AFB per 100 oil immersion fields	POS	1+	100
1-9 AFB per 100 oil immersion fields	POS	Scanty-B*	100
No AFB in 100 oil immersion fields	NEG		100

* Record actual number of bacilli seen in 100 fields – e.g. “Scanty 4”

Table 2: Consistency & classification of errors in quality assurance of sputum smear microscopy[3]

DTC reader’s smear result	IRL Microbiologist’s smear results				
	Negative	Scanty	1+	2+	3+
Negative	Correct	LFN	HFN	HFN	HFN
Scanty	LFP	Correct	Correct	QE	QE
1+	HFP	Correct	Correct	Correct	QE
2+	HFP	QE	Correct	Correct	Correct
3+	HFP	QE	QE	Correct	Correct

Correct = True, No Error
High False Positive (HFP) = negative smear reported as 1+, 2+, or 3+
High False Negative (HFN) = 1+, 2+, or 3+ smear reported as negative
Low False Positive (LFP) = negative smear reported as scanty
Low False Negative (LFN) = scanty smear reported as negative
Quantification Error (QE) = positive smear reported as positive, but outside $\pm$ 1 range

RESULTS

IRL visited 32 centres in the first EQA OSE round and 30 centres in the second EQA OSE round. A total of 153 and 142 readers were evaluated in the first and the second rounds respectively with the help of panel slides. Thus a total of 62 centres were visited and 295 readers (STLSs and DTC-LTs) were evaluated. On each occasion all readers were evaluated using a set of five panel slides prepared by the IRL. The proportion of positive/negative slides was significantly different (p=0.00013) in the two rounds of Proficiency Panel Testing as 3.7: 1.3 and 3.2: 1.8 in the first and the second rounds respectively. Overall, 1475 panel testing slides were used; 765 in the first round and 710 in the second rounds as can be seen in Table 3.

Nine readers in each round reported any type of error, amounting approximately 5% of the total readers. We did not find any High False Positive (HFP) error in the present study. One High False Negative (HFN) error was observed in the first OSE round. One Low False Positive (LFP) error was encountered during the first round but this DTC was not visited in the second round. Four Low False Negative (LFN) errors were observed; three during the first round and one during the second round. A total of 12 Quantification Errors (QE) were observed; four during the first round and eight during the second round. However, none of the proportions of the errors were significantly different when compared between the two rounds (p more than 0.05) as can be seen in Table 3.

Table 3: Descriptive characteristics

Details	First Round	Second Round	Z Test
Total number of DTC visited	32	30 *	
Total number of STLS/ DTC-LT undergoing Proficiency Panel Testing	153	142	
Total number of STLS/ DTC-LT trained in the RNTCP as on the day of Proficiency Panel Testing	150	124	P< 0.05
Total number of slides used for Proficiency Panel Testing	765	710	
Total number of Positive slides used for Proficiency Panel Testing	563	457	P< 0.05
Total number of Negative slides used for Proficiency Panel Testing	202	253	P< 0.05
Readers with High False Positive errors (HFP)	0	0	
Readers with High False Negative errors (HFN)	0.7%	0.0%	P> 0.05
Readers with Low False Positive (LFP) errors	0.7%	0%	P> 0.05
Readers with Low False Negative (LFN) errors	2.1%	0.7%	P> 0.05
Readers with Quantitative Errors (QE)	2.6%	4.2%	P> 0.05
Readers with any errors	5.9%	4.9%	P> 0.05
Total number of High False Positive errors (HFP)	0	0	
Total number of High False Negative errors (HFN)	1	0	P> 0.05
Total number of Low False Positive (LFP) errors	1	0	P> 0.05
Total number of Low False Negative (LFN) errors	3	1	P> 0.05
Total number of Quantitative Errors (QE)	4	8	P> 0.05
Total number of errors (Any error)	9	9	P> 0.05

* District TB Centres of Silvassa & Daman were not visited in second round.

Table 4: Z-N grade agreement of each sputum smear in two rounds of IRL EQA OSE visits

IRL OSE Round	DTC reader's smear result	IRL Microbiologist's smear results					
		Negative	Scanty	1+	2+	3+	Total
First	Negative	200	3	1	0	0	204
	Scanty	1	68	2	0	0	71
	1+	0	63	83	9	0	155
	2+	0	2	61	99	6	168
	3+	0	0	2	43	122	167
	Total	201	136	149	151	128	765
Second	Negative	253	1	0	0	0	254
	Scanty	0	71	4	1	0	76
	1+	0	89	127	3	0	219
	2+	0	6	54	37	4	101
	3+	0	0	1	19	40	60
	Total	253	167	186	60	44	710

It can be seen from Tables 4 and 5 that overall consistency level between the IRL Microbiologist and district reader was more than 98% during both the OSE rounds and for all grades of sputum slides (p more than 0.05). However, level of consistency was significantly different (p= 0.0002) between scanty slides and other grade slides, being least in scanty slides around 96% as compared to other grade slides averaging over 99-100%.

Tendency to under-grade or over-grade the Proficiency Panel Test slides for "erroneous slides" had insignificant difference between the two rounds. However, the tendency to over-grade the panel slides was much higher (more than 22%) as compared to under-grade (less than 2%) them in "correct slides". This over-grading tendency by district level readers might be of critical significance as 11 out of 12 QEs can be associated with it. Interestingly, exact gradation of Proficiency Panel Test slides by the district readers was high with concordance level of more than 75% during both the rounds, which can be seen in Table 6.

Around 13% of the readers in the second IRL, EQA, OSE round were untrained as compared to

Table 5: Consistency of results in different grades of Panel Test slides

Consistency of results by DTC readers as compared to IRL results	First Round	Second Round	Z Test
Consistency in Negative slides	99.5%	100.0%	P> 0.05
Consistency in Scanty slides	96.3%	95.8%	P> 0.05
Consistency in 1+ slides	98.0%	99.5%	P> 0.05
Consistency in 2+ slides	100.0%	99.5%	P> 0.05
Consistency in 3+ slides	100.0%	100.0%	P> 0.05
Consistency in ***all*** Positive slides	98.6%	98.0%	P> 0.05
Overall Consistency (all slides)	98.6%	98.2%	P> 0.05

Table 6: Tendency to under- grade or over- grade the Proficiency Panel Test slides by DTC readers during panel testing rounds

Tendency	First Round	Second Round
Erroneous Slides as per classification		
Grading less than actual / Under- grading	0.5%	0.1%
Grading more than actual / Over-grading	0.7%	1.0%
Correct Slides as per classification		
Grading less than actual / Under- grading	22.1%	23.2%
Grading more than actual / Over-grading	2.0%	1.0%
Exact grading	75.9%	75.8%

2% in the first IRL, EQA, OSE round (p= 0.00035). However, analysis of Z-N grade agreement does not suggest appreciable difference between training status of readers as can be seen in Table 7.

DISCUSSION

Proficiency Panel Testing assesses STLS's technical performance, including Ziehl-Neelsen stain preparation, Z-N staining technique, smear reading, and reporting skills. This helps Intermediate Reference Laboratory determine the source of performance problems and take remedial actions. Overall 95% of the readers (STLS/ DTC-LTs) undergoing Panel Testing during the study had reported excellent proficiency and no error. Though this system does not assess the routine laboratory performance, it is a good indicator of quality of technical proficiency of these laboratory supervisors and is of paramount importance in multi-layer quality assurance system in the RNTCP

Table 7: Z-N grade agreement in two rounds of IRL EQA OSE visit as per training status of DTC readers

Trained in the RNTCP	DTC reader's smear results	IRL Microbiologist's smear results					
		Negative	Scanty	1+	2+	3+	Total
Yes	Negative	424	3	1	0	0	428
	Scanty	1	128	5	1	0	135
	1+	0	146	196	12	0	354
	2+	0	8	109	137	12	266
	3+	0	0	3	57	127	187
	Total	425	285	314	207	139	1370
No	Negative	29	1	0	0	0	30
	Scanty	0	11	1	0	0	12
	1+	0	6	14	0	0	20
	2+	0	0	6	12	1	19
	3+	0	0	0	7	17	24
	Total	29	18	21	19	18	105

sputum smear microscopy. Similar results have been observed in the quality assurance studies in eight state tuberculosis laboratories in India with overall average consistency of 90-100% in most of the laboratories[6]. Overall high consistency of more than 98% in both the OSE rounds demonstrates the reproducibility and reliability of the Proficiency Panel Testing as an important tool of External Quality Assessment. This study confirms the importance of On-Site field visit as an ideal way to obtain a realistic assessment of the conditions and skills practised in the laboratory, and hence act, as an essential component of Quality Assurance programme[6]. Majority of laboratory staff were retained over the study period. Untrained/newly recruited laboratory supervisors did not deteriorate proficiency results in the second round. This can be attributed to their skill development during routine supervision and good laboratory practices. This indirectly depicts the strength of the RNTCP as a public health programme ensuring swift implementation of quality sputum microscopy services in Gujarat. Consistency in reading AFB negative slides by DTC readers was better as compared to AFB positive slides, with overall consistency level of more than 98%. This level is higher than National Tuberculosis Institute, Banglore study which had reported overall consistency level of around 80%[7]. Higher consistency observed in this study is due to intense supervision and monitoring as well as skill development and stricter implementation of quality assurance protocol in the relatively good performing state of Gujarat. In comparison to the first EQA, OSE round, the proportion of higher grade positive slides (3+ and 2+) was reduced in the second EQA, OSE round; and the proportion of lower grade positive slides (1+ and scanty) and negative slides was raised. A comparatively higher proportion of quantitative errors in the second OSE round may be the reflection of this reallocation in addition to the over-grading tendency of distinct level readers. Thus, Proficiency Panel Testing can be made more effective by raising the proportion of low grade positive slides in panel set of each reader. DTC reader's overall agreement level of more than 95% in Z-N grade suggests high level of precision and excellent consistency during both the IRL, EQA, OSE rounds. **It is concluded that even for a large network of sputum smear microscopy centres under public health programmes like the RNTCP in order to take corrective action, Proficiency Panel Testing can be effectively used for quick identification of suboptimal- technical performance of the supervisory staff.**

ACKNOWLEDGEMENTS

Authors gratefully acknowledge all staff of IRL, Ahmedabad for assisting in preparing the Panel Testing slides and DTOs of Gujarat and UT for support during the IRL-OSE visits to the districts in conducting the panel testing of STLSs and DTC-LTs.

REFERENCES

1. World Health Organization. Framework for effective tuberculosis control. WHO/TB/94.179. Geneva: WHO, 1994.
2. Association of Public Health Laboratories, Centres for Disease Prevention and Control, International Union Against Tuberculosis and Lung Disease, KNCV, Research Institute of Tuberculosis, and World Health Organization. External quality assessments for AFB smear microscopy. Washington DC, USA: Association of Public Health Laboratories, 2002.
3. Central TB Division. Directorate General of Health Services, Ministry of Health and Family Welfare. RNTCP laboratory network guidelines for quality assurance of smear microscopy for diagnosing tuberculosis. New Delhi, India: CTD, 2005.
4. State TB Cell. TB Gujarat, Annual status report- 2010. Gandhinagar, Gujarat: March 2010. p 16
5. Central TB Division. Directorate General of Health Services, Ministry of Health and Family Welfare. RNTCP Training Module for Laboratory Technicians. New Delhi, India: CTD, October 2005.
6. Paramasivan C. N., Venkatraman P., Vasanthan J. S., Rahman F., Narayanan P. R. Quality assurance studies in eight state tuberculosis laboratories in India, *Int J Tuberc and Lung Dis* 2003; **7(6):**522-7.
7. Ajaykumar T, Shilpa S, Shyni S, Balasangameshwara VH & Kumar P. Efforts of NTI as a national reference laboratory for the implementation of External Quality Assessment for the sputum microscopy in the ten allotted states during 2005-06, NTI Bulletin 2006, **42/1&2**, 15-24

Case Report

ROLE OF ENDOSCOPIC ULTRASOUND GUIDED FNAC IN DIAGNOSIS OF PANCREATIC TB PRESENTING AS MASS LESION: A CASE REPORT AND REVIEW OF LITERATURE

Pankaj Gupta[1], Sandeep Guleria[1**] and Shipra Agarwal[2*]**

(Received on 13.1.2011. Accepted after revision on 15.6.2011)

***Summary*:** A 24-year-old male patient presented with abdominal pain, obstructive jaundice, anorexia and weight loss. Ultrasound abdomen revealed pancreatic head mass with dilated common hepatic duct and intrahepatic bliliary radicles. CECT abdomen was suggestive of pancreatic head mass invading portal vein, splenic artery and hepatic artery. Provisional diagnosis of unresectable carcinoma head of pancreas was established. Endoscopic ultrasound (EUS) was done, which was also suggestive of pancreatic head mass infiltrating portal vein. EUS guided Fine Needle Aspiration Cytology (FNAC) was taken with an intent to obtain tissue diagnosis and to start palliative chemotherapy. EUS guided FNAC features were suggestive of tuberculosis (TB). Patient was started on anti-tubercular therapy, to which he responded and was cured. Pancreatic tuberculosis should be considered as a possibility, in pancreatic mass, especially in countries where TB is endemic and establishing its diagnosis with the aid of FNAC can save trauma of major surgery to the patient, which prompted us to report this case.

[*Indian J Tuberc 2011; 58:* 120-124]

***Key words*:** Pancreatic Tuberculosis, Endoscopic ultrasound, Pancreatic mass

INTRODUCTION

TB is endemic disease in India and with the advent of the Acquired Immune Deficiency Syndrome (AIDS), its incidence in the developed countries is also on the rise. Extra-pulmonary TB accounts for 10-15 per cent of all cases, but this incidence reaches 50% in patients with AIDS. Gastro-intestinal Tract (GI) is the sixth most frequent site of extra pulmonary TB after lymphatic, genitourinary, bone and joint, miliary and meningeal tuberculosis. Abdominal tuberculosis accounts for 0·8% of all hospital admissions in India. TB can involve any part of the gastrointestinal tract. 85 % of abdominal TB cases manifest in the ileocecal region, followed by the ascending colon, jejunum, appendix, duodenum, stomach, sigmoid colon and rectum. Pancreatic TB is very rare clinical entity and usually occurs as a part of disseminated disease, with reported incidence varying from 2 % to 4.7% in cases of miliary TB.[1,2] It most commonly presents as pancreatic mass mimicking malignancy, leading to "avoidable" major surgery. A case of pancreatic tuberculosis, which was diagnosed by EUS, guided FNAC and treated non-operatively with anti-tubercular therapy, is reported here .

CASE REPORT

A 24-year-old male patient presented with complaints of progressively worsening epigastric pain, increasing jaundice, pruritus, pale stool, loss of appetite, weight loss and low grade fever for the last two months. There was no history of cough, hemetemesis, haemoptysis, maelena or past history of tuberculosis. Physical examination revealed moderate jaundice and palpable soft gall-bladder without any lymphadenopathy. Laboratory investigations confirmed obstructive jaundice with serum bilirubin level of 22 mg/dl, elevated alkaline phosphatase140 Bodansky unit/L (normal 1.5-40 unit/L), and slightly elevated transaminase level. CA-19.9 level was normal. He was HIV negative. His chest radiograph was normal. Abdominal ultrasound showed ill-defined hypoechoic mass measuring 3.5x2.5 cm in head of pancreas with dilated common bile duct (CBD), measuring 2cm and dilated intrahepatic biliary

1. Senior Resident 2. Professor
Departments of Pathology* and Surgical Disciplines**, All India Institute of Medical Sciences, New Delhi.
Correspondence: Dr. Sandeep Guleria, Professor ,Department Of Surgical Disciplines, 5th floor, Teaching block, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India-110029; Phone: 91-9891640002; Email: sandeepguleria@hotmail.com

radicles (IHBR). Contrast enhanced computerized tomography (CECT) abdomen revealed 4 x 2.5 cm mass in head of pancreas infiltrating the portal vein, splenic artery and hepatic artery, suggestive of pancreatic malignancy (Figure 1). Side viewing Upper GI endoscopy was normal. EUS was planned, which revealed 4x 2.5 cm hypervascular mass in head of pancreas, extending into body. Mass was seen infiltrating portal vein, splenic and hepatic artery and also blocking the dilated CBD. There was no significant retroperitional lymphadenopathy, FNAC was done from the mass under EUS guidance, which revealed necrotizing epithelioid cell granulomas suggestive of TB (Figure 2). CBD stenting was done with 10Fr x 10cm stent to relieve obstructive jaundice and patient was started on four drug anti-tubercular therapy with Isoniazid, Rifampicin, Ethambutol and Pyrazinamide. For the last eight months, patient has been on our follow up and he responded to the treatment and mass has decreased considerably (Figure 3).

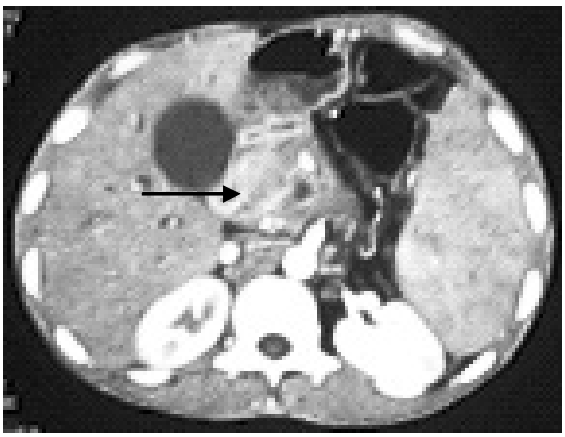


Figure 1: CECT Abdomen showing heterogeneous pancreatic head mass (arrow) deforming the superior mesenteric vein (SMV) and with no definite fat planes with superior mesenteric artery (SMA).

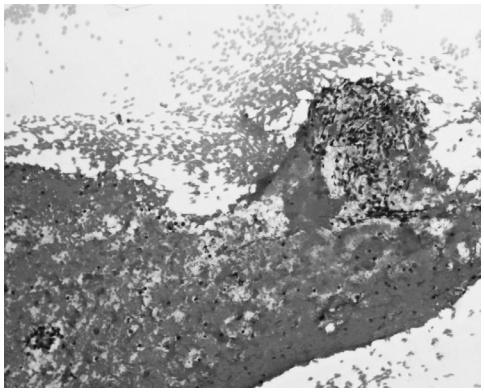


Figure 2: Photomicrograph showing normal pancreatic tissue in the form pancreatic duct (on the left side), along with an epithelioid cell granuloma (H&E, X10)

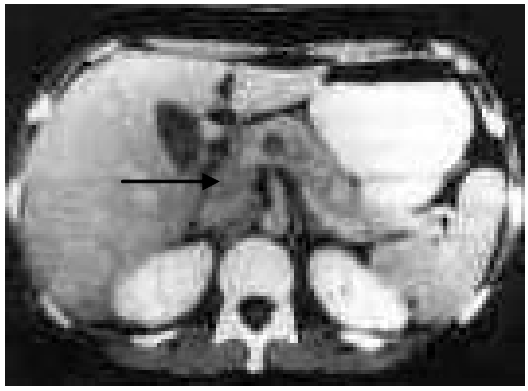


Figure 3: CECT Abdomen done at eight months showing considerable reduction in mass lesion

DISCUSSION

TB is an important medical, social and economic problem in developing countries. According to the World Health Organization (WHO), in India alone, there are 3–4 million new cases of TB every year, 2–5% of these are abdominal. It is estimated that there would be approximately 100–200,000 cases of abdominal TB in India every year. The commonest site for gastro-intestinal TB is the ileo-caecal region. Pancreatic TB is uncommon even in parts of the world where TB is endemic and isolated involvement is very rare.[3,4] Auerback *et al* reviewed 1656 autopsies performed on TB patients

and did not find any cases of isolated TB of the pancreas[1]. Bhansali *et al* found no cases of pancreatic TB in a series of 300 cases of abdominal tuberculosis in India[5]. M.T. Taher *et al* reported a case of isolated Pancreatic TB. The patient presented with epigastric pain, anorexia and weight loss. CECT Abdomen showed a heterogeneous mass with necrosis in the head and body of the pancreas and peripancreatic lymphadenopathy. Biopsies of the pancreas and lymph nodes showed inflammatory changes with multiple granuloma and caseous necrosis suggestive TB. The patient was treated with Anti-tubercular Therapy (ATT).[6] Watanapa *et al* published a case of obstructive jaundice caused by a tuberculosis of pancreas.[7] Imaging studies were suggestive of pancreatic carcinoma. Pylorus-preserving proximal pancreatoduodenectomy was performed. Histopathological examination of resected specimen revealed tuberculosis of the pancreas. Radoje Colovic *et al* reported a case of Pancreatic TB, the patient presented with abdominal pain, obstructive jaundice, malaise and weight loss. Abdominal Ultrasonography, CT abdomen and ERCP were suggestive of stenosis of the distal common bile duct, caused by a mass in the head of the pancreas. At operation, an enlarged, centrally caseous lymph node of the posterior head of the pancreas was found, causing inflammatory stenosis and a fistula with the distal CBD. The lymph node was removed and the bile duct resected and anastomosed with the Roux-en Y jejunal limb. Histology confirmed tuberculous lymphadenitis. The patient was treated with anti-tuberculous medication.[8] Sundeep S. Saluja *et al* published a series of seven patients with pancreatic TB.[9] They suggested that diagnosis of TB should be considered as a differential diagnosis, particularly in young patients, coming from areas where tuberculosis is endemic and preoperative tissue and/or cytological diagnosis should be attempted before labelling them as malignancy. Pancreatic TB usually occurs in immunocompromised patients, especially those with HIV. Shyam Dang *et al* reported a case of Pancreatic TB in HIV positive patient.[10] The patient presented with epigastric pain. CT scan of abdomen showed large necrotic node on the posterior aspect of the head of pancreas and multiple cystic masses adjacent to the pancreas. Acid fast bacilli were found on staining of CT guided biopsy of the node. Cultures grew *Mycobacterium tuberculosis*. ATT was initiated and resulted in gradual resolution of symptoms.

Pancreatic TB is rare probably due to pancreatic enzymes, which interfere with seeding of *Mycobacterium tuberculosis*.[11] Pancreatic TB occurs either by haematogenous spread in cases of miliary TB or by direct spread from contagious lymph nodes. Presentation of pancreatic TB is varied and non-specific. In a series of 16 cases of pancreatic TB by Xia *et-al*, abdominal pain was most common symptom in 75% cases followed by anorexia and weight loss in 69% cases, malaise and weakness in 64% and fever and night sweats in 50% cases.[12] It may also present as obstructive jaundice, massive gastro-intestinal bleeding, acute pancreatitis, chronic pancreatitis, pancreatic abscess or secondary diabetes.

Due to lack of pathognomonic findings of pancreatic TB on radiological studies (CECT, endoscopic ultrasound, etc.), most of the times patients are subjected to major "avoidable" surgery. Ultrasonography may reveal hypo-echoic or cystic lesion. CECT abdomen shows hypodense lesions with irregular borders mostly in pancreatic head, diffuse enlargement of pancreas or peripancreatic lymphadenopathy with ring enhancement.[13] However, these findings are non-specific and may be seen in focal pancreatitis or pancreatic carcinoma. Features on MRI are hypo-intense lesions on fat-suppressed T1-weighted images and a mixture of hypo- and hyper intense on T2-weighted images.[14] ERCP demonstrates a normal pancreatogram with a smooth narrowing of the CBD. FDG-PET scanning is not useful in distinguishing TB from pancreatic malignancy, as there is increased uptake of the FDG metabolite in both conditions.[15]

The definitive diagnosis rests on histological and bacteriological evidence of tuberculosis. Pancreatic TB is usually not suspected pre-operatively and patient is subjected to "avoidable major surgery". However if Pancreatic TB is suspected, image guided biopsy of lesion is an useful tool. In literature, only few cases have been diagnosed pre-operatively by biopsy. Sanjay D'Cruz *et al* reported a case of pancreatic T.B, where patient presented with pancreatic pain. Ultrasound of the abdomen revealed

a mass in the region of the head of the pancreas which was confirmed on CT scan of the abdomen. Diagnosis of recurrent pancreatitis with malignant pancreatic mass was made at the stage. Ultrasound guided FNAC was done, which revealed caseating granulomas suggestive of Pancreatic TB. The patient was treated successfully by ATT. [16] Schneider *et al* published two cases of Pancreatic TB mimicking carcinoma on CT scan. In the first case, explorative laparotomy revealed granulomatous inflammation suggestive of tuberculosis. The patient responded well to ATT. In the second case, diagnosis of Pancreatic TB was established by means of image guided percutaneous biopsy.[17] Pramesh *et al* published two cases of Pancreatic TB. Diagnosis was established after laparatomy in one case and by pre-operative CT guided FNAC in another.[18] Tan *et al* reported series of three cases, of pancreatic TB that masqueraded as malignancy. In all three cases, computed tomographic scan was suggestive of pancreatic carcinoma. Two patients underwent laparatomy whereas one was diagnosed by CT guided percutaneous biopsy [19]. Success rate of image guided percutaneous biopsy of pancreatic lesion is very poor, less than 50%.

EUS, a technique combining endoscopic and ultrasound images, is being increasingly used to obtain sample for diagnosis from pancreatic mass, and is considered investigation of choice for pancreatic mass. EUS-FNAC has proved to be an excellent tool for the cytological diagnosis of pancreatic and peripancreatic masses. A definitive cytological diagnosis is possible by EUS-FNAC in 80% to 95% of cases. Giovannini *et al* reported accuracy of 79 % of EUS guided biopsy in diagnosis of pancreatic masses[20], whereas Faigel *et al*[21] reported accuracy of 96%. Ahlawat *et al* reported a case of pancreatic tuberculosis who presented with fever of undetermined origin and a pancreatic mass on CT scan. EUS also demonstrated a pancreatic body mass of heterogeneous echotexture and ill-defined margins. EUS guided FNAC was taken which revealed features suggestive of pancreatic T.B.[22] Shabbir Asim *et al* published a case of pancreatic tuberculous abscess.[23] Patient presented with pyrexia, and was found to have cystic pancreatic mass on CT scan, encasing common hepatic artery, suggestive of pancreatic malignancy. Pancreatic tuberculous abscess was diagnosed by EUS-guided FNAC and patient was treated with ATT and thereby major surgery was averted. Borentain *et al* reported a case of pancreatic TB. The patient presented with obstructive jaundice. Diagnosis of Pancreatic TB was made by EUS guided biopsy of the pancreatic mass lesion.[24] EUS-FNAC is a safe, quick, radiation-free, and relatively painless way of obtaining tissue for staining, cytology and culture. The risk of acute pancreatitis ranges from 1 to 2%. Other complications like bleeding or peritonitis are also rare.

Other methods of diagnosis include cytology of bile sample obtained by ERCP and histological examination of tissue obtained by diagnostic laparoscopy. Most cases reported in literature have been diagnosed only after laparotomy, and resection of mass lesion, considering it to be neoplastic. Ahchong *et al* reviewed 12 reported cases of pancreatic TB, in 10 cases, diagnosis was made post-operatively, and in only two cases, diagnosis could be made without exploration by FNAC. [25] In our case also, our working diagnosis was carcinoma pancreas, and biopsy was undertaken, as it seemed to be unresectable lesion on radiology (CECT and endoscopic ultrasound), with a plan to start palliative chemotherapy after obtaining tissue diagnosis.

Prognosis of pancreatic TB is good, once a diagnosis is established. Anti-tubercular therapy cures disease in almost all cases. Zuber Ahmad *et al* reviewed 14 cases of pancreatic tuberculosis, 11 patients were given ATT, 10 patients showed good response, there was one mortality due to delay in diagnosis. [26] Beaulieu *et al* also reported a case of successful treatment of pancreatic TB with quadruple ATT.[27] Patients with features of biliary obstruction may require endoscopic stenting, as was required in our case. Obstructing lymph node may require surgical excision.

CONCLUSIONS

In view of the non-specific presentation and imaging appearance of the disease, a high index of suspicion is required to obtain a pre-operative diagnosis. The diagnosis of abdominal TB should be considered in the context of a mass in the head of the pancreas in the

immunocompromised patients and in countries with endemic TB. The definitive diagnosis rests on histological and bacteriological evidence of tuberculosis. EUS or CT-guided FNAC is recommended for diagnosis. The prognosis is good with ATT.

REFERENCES

1. Auerbach O. Acute generalized miliary tuberculosis. *Am J Pathol* 1944; **20**: 121-6.
2. Paraf, Menager C, Texier J. La tuberculose du pancreas et la tuberculose des ganglions de l'etage superieur de l'abdomen. *Rew Med Chir Mal Foie* 1996; **41**: 101-26.
3. Leder RA, Low VHS. Tuberculosis of the abdomen. *Radiol Clin N Am* 1995; **33**: 691-705.
4. Jadaver H, Mindelzun RE, Olcott EW, Levitt DB. Still the great mimicker: abdominal tuberculosis. *AJR* 1997; **168**: 1455-60.
5. Bhansali SK. Abdominal tuberculosis experience with 300 cases. *American journal of gastroenterology* 1977; **67**: 324-37.
6. M.T. Taher,1 S.M.H. Hashemi,1 M. Mohammadi 1 and F. Hashemi. Tuberculosis of pancreas and peripancreatic lymph nodes: a case report. *Eastern Mediterranean Health Journal* 2009; **15(6)**: 1617-20.
7. P. Watanapa and V. Vathanopas. Tuberculous pancreatic abscess: a rare condition mimicking carcinoma. *HPB Surgery*1992; **5**: 209-13.
8. Radoje Colovic, Nikica Grubor, Rada Jesic, Marjan Micev, Tanja Jovanovic, Natasa Colovic, Henry Dushan Atkinson. Tuberculous lymphadenitis as a cause of obstructive jaundice: A case report and literature review. *World J Gastroenterol* 2008; **14(19)**: 3098-100.
9. Sundeep S Saluja, Sukanta Ray, Sujoy Pal, Manu Kukeraja, Deep N Srivastava, Peush Sahni and Tushar K Chattopadhyay. Hepatobiliary and pancreatic tuberculosis: A two decade experience. *BMC Surgery* 2007; (**7**): 1471-2482.
10. Shyam Dang, Muslim Atiq, Michael Saccente, Kevin W Olden, Farshad Aduli. Isolated Tuberculosis of the Pancreas: A Case Report. *J Pancreas (Online)* 2009; **10(1)**: 64-6.
11. Franco-Paredes C, Leonard M, Jurado R, Blumberg HM, Smith RM. Tuberculosis of the pancreas: report of two cases and review of the literature. *Am J Med Sci* 2002; **323**: 54-8.
12. Xia F, Poon RT, Wang SG, Bie P, Huang XQ, Dong JH.Tuberculosis of pancreas and peripancreatic lymph nodes inimmunocompetent patients: experience from China. *World J Gastroenterol* 2003; **9**: 1361-64.
13. Pombo F, Diaz-Candamio MJ, Rodriguez E, Pombo S. Pancreatic tuberculosis: CT findings. *Abdom Imaging* 1998; **23**: 394-7.
14. De Backer, K. J. Mortelé, P. Bomans, B. L. De Keulenaer, I. J. Vanschoubroeck and M. M. Kockx. Tuberculosis of the Pancreas: MRI Features. *AJR* 2005; **184**: 50-4
15. Obama K, Kanai M, Taki Y, Nakamoto Y, Takabayashi A. Tuberculous lymphadenitis as a cause of obstructive jaundice: report of a case. *Surg Today* 2003; **33**: 229-31.
16. Sanjay D'Cruz, Atul Sachdev, Ladbans Kaur, Uma Handa, Ashish Bhalla, Sarabmeet S Lehl. Fine Needle Aspiration Diagnosis of Isolated Pancreatic Tuberculosis: A Case Report and Review of Literature. *J Pancreas (Online)* 2003; **4(4)**: 158-62.
17. Schneider A, von Birgelen C, Duhrsen U, Gerken G, Runzi M. Two cases of pancreatic tuberculosis in nonimmunocompromised patients. A diagnostic challenge and a rare cause of portal hypertension. *J Pancreas (Online)* 2005; **6(6)**: 598-602.
18. CS Pramesh, AA Heroor, SG Gupta, S Krishnamurthy, PJ Shukla, P Jagannath, and LJ Desouza. Pancreatic tuberculosis: an elusive diagnosis. *HPB* 2003; **5(1)**: 43-5.
19. Tan, Ker-Kan, Chen, Kenneth, Liau, Kui-Hin, Ho, Choon-Kiat. Pancreatic tuberculosis mimicking pancreatic carcinoma: series of three cases. *European Journal of Gastroenterology & Hepatology* 2009; **21(11)**: 1317-19.
20. Giovannini M, Seitz JF, Monges F, Perrier H, Rabbia I. Fine-needle aspiration cytology guided by endoscopic ultrasonography: results in 141 patients. *Endoscopy* 1995; **27**: 171-7.
21. Faigel DO, Ginsberg GG, Bentz JS, Gupta PK, Smith DB, Kochman ML. Endoscopic ultraosound-guided real-time fine-needle aspiration biopsy of the pancreas in cancer patients with pancreatic lesions. *J Clin Oncol* 1997; **15**: 1439-43.
22. Sushil K Ahlawat, Aline Charabaty-Pishvaian, James H Lewis, Nadim G Haddad. Pancreatic Tuberculosis Diagnosed with Endoscopic Ultrasound Guided Fine Needle Aspiration. *J Pancreas (Online)* 2005; **6(6)**: 598-602.
23. Shabbir Asim, Lahiri Manjari, Mak Seck-wai Kenneth, Chang Russell Alexander, Khor Christopher, Khek-Yu Ho. Pancreatic tuberculous abscess diagnosed by endoscopic ultrasound-guided fine needle aspiration. *JPMA* 2010; **60(6)**: 499-501.
24. Borentain P, Grandval P, Ananian P, Meunier-Carpentier S, Gérolami R, Escoffier JM, Durand JM, Botta-Fridlund D, Bernardini D. Pancreatic tuberculosis: an unusual cause of obstructive jaundice accessible to endoscopic management. *Gastroenterol Clin Biol* 2007; **31(11)**: 1024-7.
25. S. F. Lo, A. K. Ahchong, C. N. Tang, A. W. C. Yip. Pancreatic tuberculosis: case reports and review of the literature. J.R.Coll. Surg. Edinb., 43, February 1998, 65-69.
26. Zuber Ahmad, R. Bhargava, D.K. Pandey, D.K. Sharma, Mohd. Shamim. Pancreatic Tuberculosis - a case report. *Indian J Tuberc* 2003; **50**: 221.
27. Beaulieu S, Chouillard E, Petit-Jean B, Vitte RL, Eugene C. Pancreatic tuberculosis: a rare cause of pseudoneoplastic obstructive jaundice. *Gastroenterol Clin Biol* 2004; **28(3)**: 295-8.

Case Report

BILATERAL SPONTANEOUS PNEUMOTHORAX IN MILIARY TUBERCULOSIS

Manoj Arya, Jacob George, Ramakant Dixit, Rakesh C. Gupta and Neeraj Gupta

(Received on 13.4.2010. Accepted after revision on 15.6.2011)

Summary: Pneumothorax is a common complication in pulmonary tuberculosis that is usually seen with underlying cavitary lesion. However, it is uncommonly seen in patients with miliary tuberculosis. This communication describes bilateral spontaneous pneumothorax in an 18 years' old female patient having miliary tuberculosis. **[*Indian J Tuberc 2011; 58:* 125-128]**

Key words : Miliary Tuberculosis, Bilateral spontaneous pneumothorax.

INTRODUCTION

Pneumothorax as a complication of adult cavitary pulmonary tuberculosis is well known and not at all rare, however, its occurrence as a complication in miliary tuberculosis is rare[1]. A case of miliary tuberculosis complicated by bilateral spontaneous pneumothorax in a young female patient is reported here.

CASE REPORT

An 18-year-old unmaried female presented with three months' history of dry cough, evening rise fever and anorexia. For the last two days, she developed sudden breathlessness with bilateral chest pain after several bouts of dry cough. She was taking oral antibiotics and cough sedative for the last two weeks prior to admission.

The patient on presentation was in respiratory distress. She was sweating, her respiratory rate was 32 breaths per minute and pulse rate was 124 beats per minute with regular rhythm. Physical examination revealed a Body Mass Index of 18 and pallor. There was no cyanosis, icterus, clubbing, lymphadenopathy, pedal edema, etc.

On respiratory system examination, chest was bilateral symmetrical with accessory respiratory muscles working. Trachea was central and chest expansion was diminished. Percussion note was hyper resonant on both sides. Intensity of breath sound was also reduced on both sides. There were no adventitious breath sounds. Other system examination was normal.

Laboratory investigations revealed low hemoglobin (8.2 gm%) and raised ESR (42 mm at the end of one hour). Other blood investigations revealed nothing abnormal in leucocyte counts, blood sugar, and organ functions. Her HIV test was negative by ELISA method. Induced sputum was negative for acid fast bacilli. Mantoux test revealed an induration of 12 mm after 72 hours. Her ECG showed sinus tachycardia. Chest X-ray revealed diffuse miliary shadows in both lung fields with bilateral pneumothorax (Figure 1). Fundus examination showed choroid tubercles. CT scan of the chest was deferred due to poor general condition. Bed side ultrasound abdomen revealed mild hepatomegaly only.

An urgent closed tube thoracostomy with under water seal was done on left side. After thoracostomy, dyspnoea was relieved to some extent. Chest X-ray repeated on next day showed partial expansion of left lung (Figure 2). Another intercostal chest tube with under water seal was put on right side. Along with thoracostomy, patient received first line antituberculosis drugs, broad-spectrum antibiotics and

Department of Respiratory Medicine, JLN Medical College, Ajmer (Rajasthan).
***Correspondence*:** Dr Ramakant Dixit, Consultant Pulmonologist, A- 60, Chandravardai Nagar, Ajmer. -305 001 (Rajasthan); Ph: 09413040257; Email: dr.ramakantdixit@gmail.com

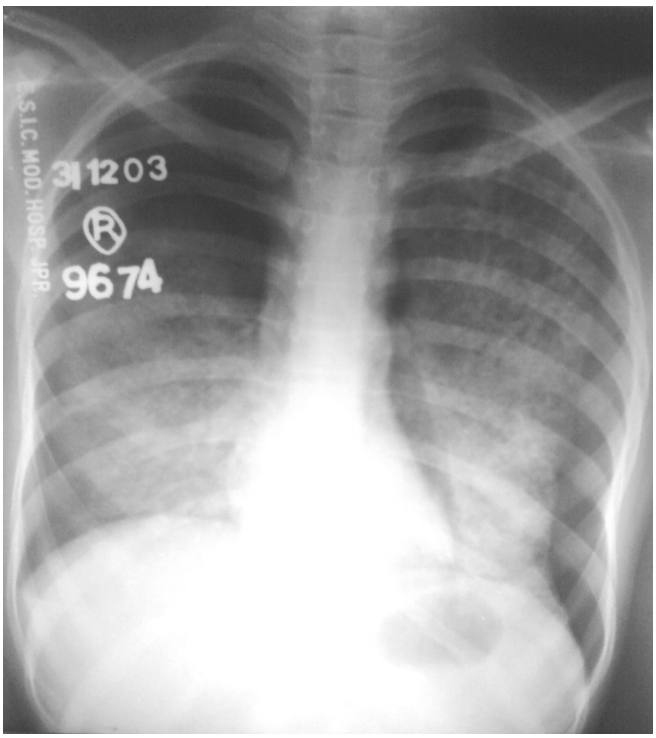


Figure 1: X-ray chest showing bilateral pneumothorax with underlying miliary tuberculosis on both sides.

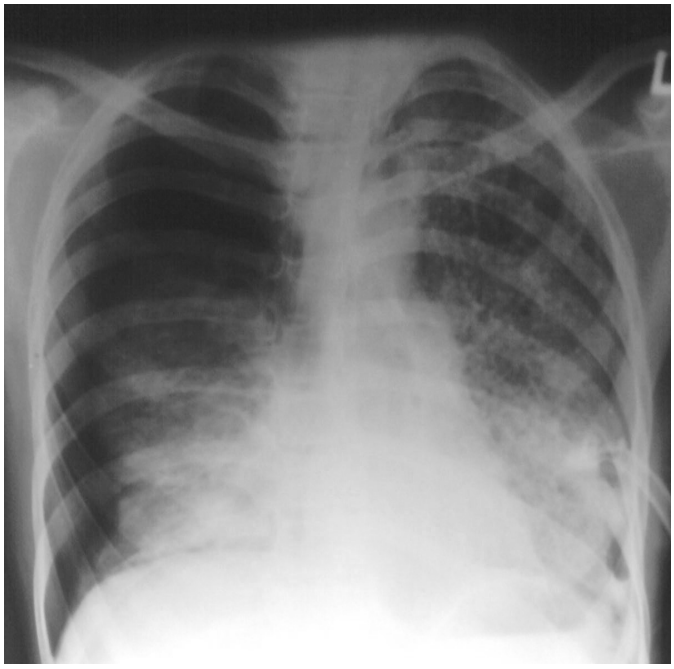


Figure 2: X- ray chest showing partial expansion of left lung with intercostal drain *in situ* and pneumothorax on right side.

oxygen inhalation that resulted in control of fever and decrease in breathlessness.

Repeated radiological examination subsequently showed complete expansion of right lung but incomplete expansion of left lung due to presence of broncho-pleural fistula. Right-sided chest tube was extubated two weeks after admission. Patient was discharged on persistent request with chest tube *in situ* because of her social problems on with anti-tuberculosis drugs and broad spectrum antibiotics. Ten days after discharge, she was again admitted in a very low general condition. Pulse was very feeble and she was gasping. Cardiopulmonary resuscitation was done but patient could not be revived.

DISCUSSION

The commonest cause of pneumothorax in our country is tuberculosis[2]. Although pneumothorax is a common complication of cavitary pulmonary tuberculosis, it is rarely seen in miliary form of the disease.

Even in patients with miliary tuberculosis, the pneumothorax has been observed mostly unilaterally. The reports of bilateral pneumothorax are very few[3.] Peiken *et al*[4] reported miliary tuberculosis in a young patient complicated by recurrent and bilateral pneumothorax. Narang *et al*[5] reported five adult cases of miliary tuberculosis, of which four were complicated by pneumothorax that was bilateral in two cases. In a study of 80 cases of pneumothorax, Agnihotri[2] found tuberculosis as a cause in 57.5% cases, of which miliary tuberculosis was responsible in 5% cases. In a series of 12 patients with simultaneous bilateral spontaneous pneumothorax, Graf-Deuel and Knoblauch[6] found miliary tuberculosis in only one case. They also reviewed 56 cases of bilateral pneumothorax in Western Literature and found miliary tuberculosis as a cause in only three cases. Thus, review of literature suggests that bilateral pneumothorax is an uncommon complication in miliary tuberculosis but carries poor prognosis with high mortality despite standard treatment[7]. The exact mechanism of pneumothorax in miliary tuberculosis is not known and various possible mechanisms have been suggested. These include:

(A) Formation of small area of confluent sub-pleural miliary nodule that undergoes caseation and necrosis with subsequent rupture into pleural space causing pneumothorax[4,5].

(B) Increased intra-alveolar pressure due to excessive coughing ruptures intra-alveolar septa that cause pneumomediastinum. Pneumothorax in this situation occurs due to rupture of air through the mediastinal pleura[8].

(C) Bullous or emphysematous lesion might form near miliary tubercles that may rupture to produce pneumothorax. This mechanism may explain the bilateral simultaneous, and/ or recurrent pneumothoraces[4, 9,10].

The first and third mechanism could be possible mechanisms in our case. A definite opinion could have been made by computer tomography, which was lacking in this case. There was no evidence of mediastinal emphysema on the available chest X-rays to support the second mechanism.

The initial treatment for pneumothorax in miliary tuberculosis is tube thoracostomy. **Open thoracostomy in miliary tuberculosis should not be considered until the patient has received antituberculosis therapy for at least several weeks. In patients with recurrent pneumothoraces, chemical pleurodesis or VATS pleurodesis may be considered for successful outcome[11]. Bilateral pneumothorax is a medical emergency that should be quickly recognized and timely managed so as to reduce mortality and morbidity.**

REFERENCES

1. Sharma N, Kumar P. Miliary tuberculosis with bilateral pheomothorax : a rare complication. *Indian J Chest Dis Allied Sci* 2002; **44**: 125-7.
2. Agnihotri S, Sharma TN, Jain NK, et al. Spontaneous pneumothorax : A clinical study of eighty cases in Jaipur. *Lung India* 1984; **4**: 189-92.
3. Chaudhry I, Bojal S, Attia A, Nwasser A. Multimodality management of extensive tuberculous bullous disease of

lung with bilateral pneumothorax. *Ann Thorac Surg* 2010; **90(1)**: 304-5.
4. Peiken AS, Lamberta F, Seriff NS. Bilateral recurrent pneumothoraces : A rare complication of miliary tuberculosis. *Am Rev Respir Dis* 1974; **110**: 512-7.
5. Narang PK, Kumar S, Gupta A. Pneumothorax and pneumomediastinum complicating acute miliary tubersulosis. *Tubercle* 1977; **58**: 79-82.
6. Graf-Deuel E, Knoblauch A. Simultaneous bilateral spontaneous pneumothorax. *Chest* 1994; **105**: 1142-6.
7. Wammanda RD, Ameh EA, Ali FU. Bilateral pneumothorax complicating miliary tuberculosis in children: case report and review of the literature. *Ann Trop Paediatr* 2003; **23(2)**:149-52.
8. Meeklin MT, Macklin CC. Malignant interstitial emphysema of the lungs and mediastinum as an important occult complication in many respiratory diseases and other conditions : An interpretation of the clinical literature in the light of laboratory experiment. *Medicine (Baltimore)* 1944; **23**: 281-358.
9. Vidyasagar B, Murali S. A case of pneumothorax complicating acute miliary tuberculosis. *Lung India* 1988; **16**: 128-9.
10. Chandra KS, Prasad AS, Prasad CE, et al. Recurrent pneumothoraces in miliary tuberculosis. *Trop Geogr Med* 1988; **40**: 347-9.
11. Liu WL, Wang HC, Luh KT, Yang PC. Recurrent bilateral pneumothoraces : a rare complication of miliary tuberculosis. *J Formos Med Assoc* 2008; **107**: 902-06.

Case Report

TUBERCULOUS OSTEOMYELITIS OF THE BONE FLAP FOLLOWING CRANIOTOMY

Arvind S Pandey[1] and Ashish Surana[2]

(Received on 7.4.2011; Accepted after revision on 15.6.2011)

Summary: A patient of tuberculous osteomyelitis of the bone flap following craniotomy for acute subdural hemorrhage which was treated at Surat Municipal Institute of Medical Education & Research (SMIMER) from June 2010 has been reported. This report emphasizes the fact that while treating osteomyelitis of bone flap following craniotomy, possibility of tuberculosis should be considered, especially in our country. Treatment wise, the disease responded readily to routine anti-tubercular chemotherapy. ***[Indian J Tuberc 2011;58: 129-131]***

Key words**:** Tuberculous osteomyelitis, Skull, Craniotomy

INTRODUCTION

Tuberculous osteomyelitis of the skull is a rare manifestation of extra-pulmonary disease[1,2]. Skeletal tuberculosis accounts for 1-3 % of all cases of tuberculosis[3] and calvarial involvement is seen only in 0.2-1.3 % of patients with skeletal TB[1].

CASE REPORT

A 20-year-old male patient with alleged history of road accident was admitted in unconscious state with history of vomiting and nasal bleeding on 28th March 2010. On examination, vitals were normal with GC Score E1V1M5. On neurological examination, there was swelling of scalp on left side and hemiparesis on right side.

Routine haematological & biochemical investigations were normal. The chest X-ray was also normal. Computerized Tomography (CT) of brain revealed acute Subdural Hemorrhage (SDH) in left fronto-temporoparietal region with significant mass effect midline shift (Figure 1). The patient was immediately subjected to left fronto-temporoparietal craniotomy and evacuation of acute SDH. The post-operative period was uneventful with no neurological deficit and patient improved well.

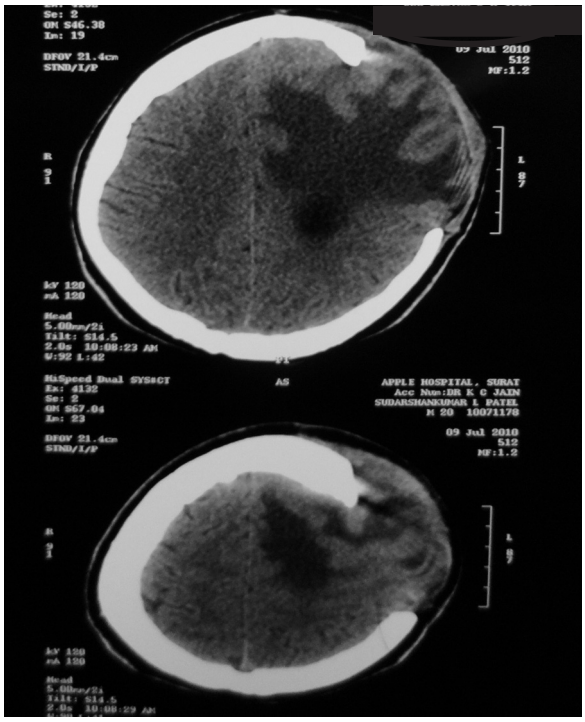


Figure 1: Post head-injury left parietal craniotomy status with scalp edema. Mild subdural haemorrhage along with falx-cerebri and tentorium cerebelli is observed

1. Professor* 2. Assistant Professor**
Departments of Microbiology** and Tuberculosis and Respiratory Diseases*, Surat Municipal Institute of Medical Education & Research (SMIMER), Surat, Gujarat, India
Correspondence**:** Dr. Ashish Surana, C-1101, Nest House Apartment, Opp DRB College, Near China Gate, New City Light, Surat-395017, Gujarat. E-mail-suranaan@yahoo.com; Phone- 09426854701 (M)

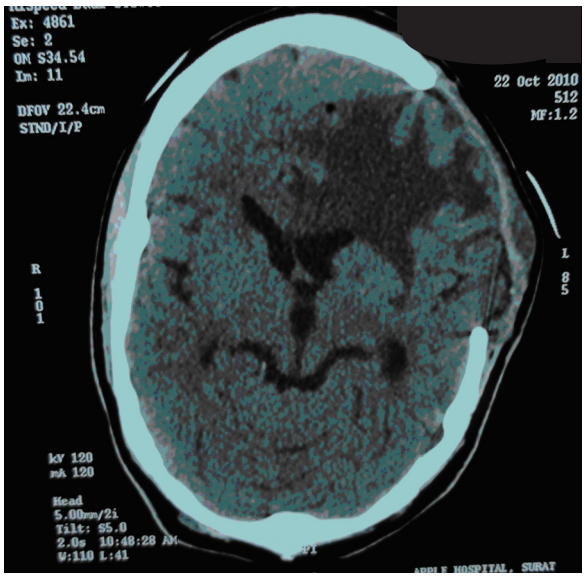


Figure 2: Development of Tuberculous Osteomyelitis in the bone flap and nereby followed by removal of the bone flap

On 3rd June 2010, approximately two months after surgery, though the patient improved with routine treatment, there was purulent discharge from the operative wound which did not subside with regular dressings and antibiotics (cefaperazone-sulbactum and netilimicin). The antibiotic therapy was based upon the culture and sensitivity testing in which Klebsiella species was reported. On examination, a sinus discharging pus was present in the surgical wound. The neurological examination was normal except diplopia.

The swelling over the local area continuously increased; hence the patient was readmitted on 19th June 2010 for debridement of the wound. During debridement, a portion of bone flap was also removed. The debrided material was sent for histopathological examination and the pus was sent for routine, fungal and AFB culture and sensitivity testing. The biopsy report was suggestive of tuberculous inflammation. The routine and fungal cultures showed no growth. The smears and culture were positive for AFB, sensitive to all the primary line of anti-tuberculous drugs. Repeat CT scan of brain was done on 28th June 2010 and showed large parenchymal hypodense area with edema in left fronto-parietal region with scalp edema and mild bony destruction with thinning of superior border of left squamous temporal bone (possibility of infective aetiology likely was reported). The patient was started on anti-tuberculous treatment as per RNTCP guidelines from the same day.

On follow up, two weeks later, in view of persisting pus discharge, another CT scan of brain was done which showed marked reduction in scalp edema and hypodense collection with mild reduction of parenchymal edema. Routine culture and sensitivity of the pus was repeated and was reported to be sterile for pyogenic organism.

Six weeks later, on 19th September 2010, the patient was readmitted with history of one episode of generalized convulsion, nausea and vomiting. During course of hospitalization, patient was treated conservatively to which the patient responded satisfactorily (Figure 2).

As on 30th November 2010, the anti-tuberculous therapy was being continued and on local examination of the wound, discharge of pus was absent with the healing of the wound almost complete. Overal, the patient was doing well, except for the history of occasional convulsions along with mild diplopia for which he was being treated conservatively.

DISCUSSION

Tuberculosis of the skull is a rare entity with occurrence of 1 in 10,000 cases of tuberculosis[3]. The majority of these cases occur at an early age with three-fourth of the patients below 20 years of age and 50% being less than 10 years of age[4]. There is no sex predilection and both sexes are almost equally affected.

In common with tuberculosis of bones and joints, lesions in the skull are almost never primary, unless there has been direct inoculation of the bone by a penetrating injury; in almost all cases, a primary lesion elsewhere in the body most commonly in the lung can be shown. Skull lesions are seen more

commonly in the fronto-parietal region than in occipito-temporal region, the ratio being five to one[5].

The tuberculous focus in the skull starts in the diploe and may erode either one or both tables of the skull, giving a clear punched out appearance on skull x ray. When the response to the infection is good, the lesion develops slowly. Wide extension of tuberculous granulation tissue through the diploe is prevented by the proliferation of and encircling layer of concentrically placed fibroblasts and if the process is not arrested, extension then takes place through either tables. If the outer table is destroyed, a fluctuating swelling of scalp develops and subsequently the skin breaks down with the formation of sinus, discharging tubercular pus. When the tissue response is poor, the infection spreads more rapidly through the dipole. The sutures form no barrier to the advance, and perforation of either tables may occur at several points. An extensive area of destruction occurs before a sinus or a fluctuating swelling appears. If the process is rapid, sequestration may occur which can take the form of so called bone sand in the punched out lesions. The dura matter forms an excellent protective barrier to the spread of the brain and meninges.

Two possible routes for origin of the infection have been suggested. Trauma has been suggested as playing a role in the genesis of this disease. Trauma by increasing the vascularity may help in localizing the lesion, to a particular part of the skull. Paucity of early symptoms is a major feature of this condition. Appearance of a fluctuating swelling of the scalp is usually the first evidence of the disease.

Treatment-wise, the disease responds very well to the usual anti-tubercular chemotherapy in the early stages. In later stages, with sequestration of bone and extensive caseation, surgical removal of all diseased tissues is essential.

CONCLUSION

It is important to consider tuberculosis as a cause of post-operative osteyomyelitis, as its treatment is quite distinct from pyogenic osteomyelitis which is the commonest cause of post-operative osteomyelitis. Hence, all bone flaps which are removed for suspected osteomyelitis should be sent for histopathological examination and for AFB and fungal cultures, in addition to routine cultures.

REFERENCES

1. Straus DC. Tuberculosis of flat bones of the vault of the skull. *Surg Gynaecol Obstet* 1933; **57**: 384-98.
2. Barton CJ. Tuberculosis of the vault of the skull. *Brit J Radiol* 1961; **34**: 286.
3. Tuli S. Epidemiology and Prevalence: tuberculosis of the skeletal system: Jaypee Brothers, New Delhi, 1993;1.
4. Schuster J, Rakasun T, Chonmaitree T. Tuberculous osteitis of skull mimicking histiocytosis. *J Pediatr* 1984; **105**: 269-71.
5. Tata RR. Tuberculosis osteomyelitis of the skull. *Indian J Tuberc* 1971; **25**: 208.

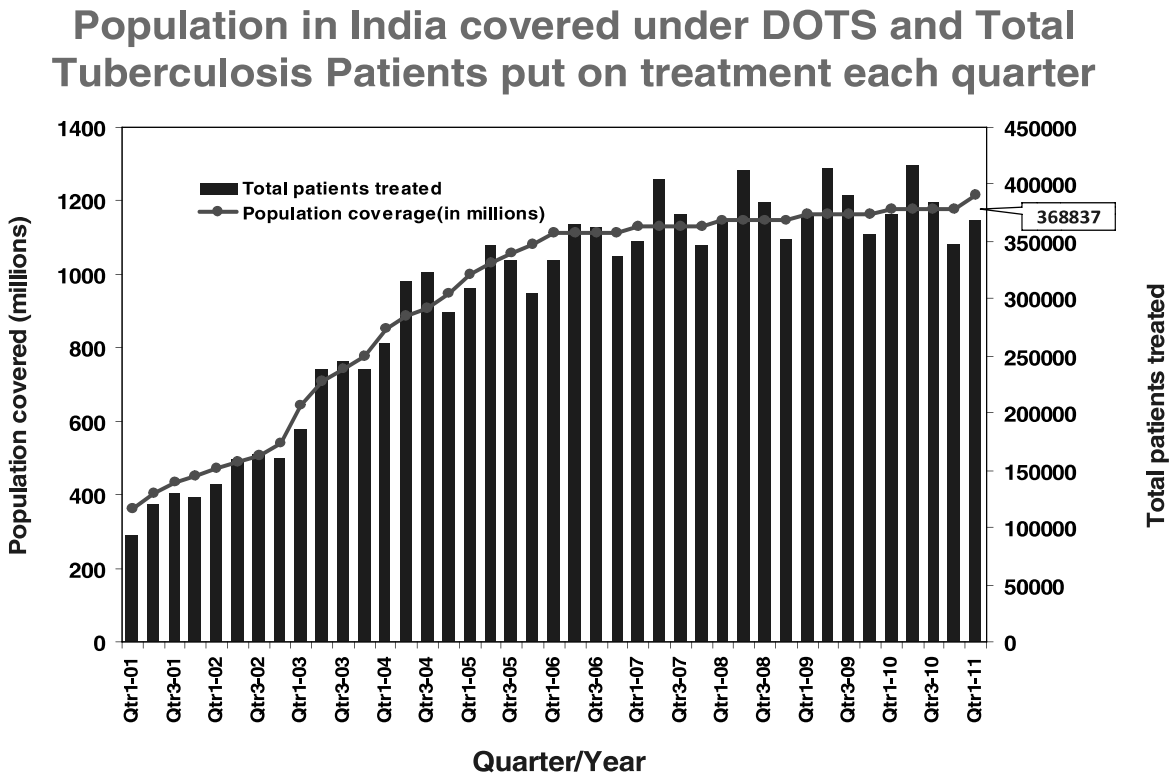
Indian J Tuberc 2011; 58: 132-135

STATUS REPORT ON RNTCP*

The RNTCP has continued to achieve the twin objectives of NSP case detection and treatment success rate at the national level during the first quarter, 2011 (Figure). 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 2011

During the quarter, over 2.03 million suspects were examined, 234,946 sputum positive cases were diagnosed, and 368,837 TB cases were registered for treatment. The annualized total case detection rate is 121 cases per 100,000 population. With a total of 156,108 new smear positive cases being registered for treatment, the new smear positive TB case notification rate (annualized) for the first quarter 2011 is 51 per lakh population. In addition to this, 83,365 new smear negative cases, 55,194 new extra pulmonary cases, 48,475 smear positive re-treatment cases and 25,220 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 2010 is 87% and the sputum conversion rate of patients registered during fourth quarter, 2010 is 91%. The default rates among NSP (5.4%), NSN (6.6%) and re-



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

Table: Performance of RNTCP Case Detection (2011, first quarter), Smear Conversion (2010, fourth quarter), and Treatment Outcomes (2010, first quarter)

State	Population (in lakh) covered by RNTCP[1]	No. of suspects examined	Suspects examined per lakh population	Rate of change in suspects examined per lakh population (compared to same quarter in previous year)	No of Smear positive patients diagnosed[2]	Suspects examined per smear positive case diagnosed	Annualized smear positive case notification rate (reported by RNTCP DMCs)	Annualized smear positive case notification rate [from CFR: sm + cases (NSP + Rel + TAD) * 4 / Pop]	Total patients registered for treatment[3]	Annualized total case notification rate	Annualized new smear positive case notification rate	Annualized new smear negative case notification rate	Annualized new extra pulmonary case notification rate
Andaman & Nicobar	5	1271	257	9%	89	14	72	62	233	189	48	57	57
Andhra Pradesh	847	150981	178	7%	20045	8	95	77	28369	134	60	32	16
Arunachal Pradesh	12	2533	204	-4%	313	8	101	86	540	174	62	37	22
Assam	306	34796	114	-2%	5050	7	66	56	8459	111	46	29	15
Bihar	980	109523	112	13%	11950	9	49	42	19162	78	34	22	5
Chandigarh	14	4376	320	0%	556	8	163	91	630	184	67	13	65
Chhattisgarh	242	28345	117	6%	3275	9	54	49	6890	114	43	43	15
D & N Haveli	4	610	172	13%	65	9	73	54	95	107	44	18	25
Daman & Diu	2	573	294	14%	37	15	76	55	75	154	43	43	18
Delhi	166	44716	269	14%	6366	7	153	123	13239	318	87	55	104
Goa	17	4372	261	31%	326	13	78	61	549	131	45	23	34
Gujarat	590	111301	189	4%	14735	8	100	82	18382	125	59	13	17
Haryana	253	41343	164	-4%	5938	7	94	72	8354	132	48	24	24
Himachal Pradesh	68	17896	263	17%	1978	9	116	98	3266	192	72	32	44
Jammu & Kashmir	117	25974	222	47%	2231	12	76	70	3249	111	58	14	21
Jharkhand	314	38494	123	12%	5729	7	73	65	9206	117	56	32	8
Karnataka	594	128178	216	10%	11259	11	76	60	17488	118	46	26	22
Kerala	346	99735	288	18%	3847	26	44	35	6694	77	31	20	18
Lakshadweep	1	138	214	141%	0		0	0	2	12	0	0	12
Madhya Pradesh	721	92646	128	14%	12836	7	71	61	21090	117	47	34	13
Maharashtra	1126	181383	161	4%	19044	10	68	59	33933	121	47	27	22
Manipur	24	3178	131	3%	316	10	52	45	673	111	37	30	24
Meghalaya	27	4779	180	-5%	597	8	90	67	1068	161	54	34	38
Mizoram	9	2062	233	28%	149	14	67	59	558	253	46	69	90
Nagaland	22	3619	162	7%	506	7	91	77	914	164	58	36	36
Orissa	408	55689	136	1%	7292	8	71	62	12090	118	52	28	21
Puducherry	12	5760	463	10%	673	9	216	65	371	119	47	25	25
Punjab	273	46384	170	8%	6290	7	92	80	9399	138	58	22	27
Rajasthan	677	97075	143	6%	16496	6	97	88	27037	160	61	39	22
Sikkim	5	1427	308	20%	157	9	136	115	307	265	85	51	66
Tamil Nadu	674	217892	323	23%	11703	19	69	60	20612	122	47	34	23
Tripura	36	5400	149	10%	503	11	56	50	708	78	43	14	10
Uttar Pradesh	2272	303737	134	-10%	46187	7	81	73	67512	119	58	26	13
Uttarakhand	99	17143	173	5%	2516	7	101	77	3453	139	54	28	23
West Bengal	889	152366	171	3%	15892	10	72	61	24230	109	49	21	18
Grand Total	**12153**	**2035695**	**168**	**6%**	**234946**	**9**	**77**	**66**	**368837**	**121**	**51**	**27**	**18**

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

State	3 month conversion rate of new smear positive patients	3 month conversion rate of retreatment patients	No (%) of all Smear Positive cases started RNTCP DOTS within 7 days of diagnosis		No (%) of all Smear Positive cases registered within one month of starting RNTCP DOTS treatment		No (%) of all cured Smear Positive cases having end of treatment follow- up sputum done within 7 days of last dose		No (%) of cases (all forms of TB) registered receiving DOT through a community volunteer		Proportion of all registered TB cases with known status	Proportion of TB patients known to be HIV infected among tested	Proportion of TB patients known to be HIV infected among registered	Proportion of HIV infected TB patients put on CPT(RT report)	Proportion of HIV infected TB patients put on ART(RT report)
Andaman & Nicobar	91%	81%	80	98%	70	85%	64	96%	34	15%	8%	0%	0%		
Andhra Pradesh	92%	76%	15086	91%	16342	98%	11151	84%	23692	84%	81%	11%	9%	91%	43%
Arunachal Pradesh	91%	77%	266	94%	276	98%	198	91%	186	34%	43%	0%	0%		
Assam	87%	65%	3723	85%	4078	94%	2897	77%	2739	32%	26%	1%	0%	100%	138%
Bihar	88%	73%	9068	88%	10050	97%	6627	81%	12247	64%	8%	4%	0%	45%	18%
Chandigarh	94%	74%	253	80%	278	88%	223	90%	135	21%	93%	1%	1%		
Chhattisgarh	89%	73%	2658	89%	2870	96%	1944	80%	3507	51%	3%	3%	0%	0%	25%
D & N Haveli	92%	53%	45	90%	47	94%	32	91%	19	20%	7%	0%	0%	50%	50%
Daman & Diu	79%	75%	16	59%	23	85%	18	78%	7	9%	65%	10%	7%	0%	33%
Delhi	91%	73%	4665	88%	5074	96%	4149	97%	895	7%	49%	2%	1%	70%	64%
Goa	89%	58%	236	90%	238	91%	180	92%	53	10%	92%	5%	4%	100%	82%
Gujarat	92%	71%	11374	92%	12190	99%	9251	90%	9859	54%	83%	5%	4%	93%	71%
Haryana	90%	77%	4249	90%	4458	94%	2913	75%	2248	27%	55%	1%	1%	0%	27%
Himachal Pradesh	92%	83%	1657	97%	1653	96%	1541	95%	424	13%	22%	2%	1%	0%	0%
Jammu & Kashmir	92%	82%	2050	98%	2075	99%	1791	91%	326	10%	2%	5%	0%		
Jharkhand	92%	79%	4499	87%	5135	99%	3138	75%	5311	58%	10%	5%	1%	0%	68%
Karnataka	89%	63%	7778	85%	8768	96%	5180	81%	8720	50%	86%	15%	13%	98%	66%
Kerala	86%	71%	2827	88%	2934	92%	2216	82%	4241	63%	40%	3%	1%	13%	16%
Lakshadweep	100%	0%	0		0		0	0%	0	0%	0%		0%		
Madhya Pradesh	91%	71%	9939	88%	11062	98%	6349	78%	12760	61%	9%	1%	0%	0%	25%
Maharashtra	90%	69%	14877	88%	16498	98%	11218	82%	9834	29%	74%	11%	8%	93%	57%
Manipur	93%	84%	260	95%	261	95%	205	83%	343	51%	44%	11%	5%	61%	48%
Meghalaya	85%	60%	426	89%	460	96%	315	82%	660	62%	10%	0%	0%		
Mizoram	90%	71%	130	96%	130	96%	99	65%	110	20%	55%	12%	6%	90%	59%
Nagaland	94%	85%	323	74%	357	82%	260	76%	408	45%	41%	5%	2%	100%	25%
Orissa	89%	65%	5350	83%	6312	98%	3817	75%	8221	68%	4%	2%	0%	0%	33%
Puducherry	89%	74%	164	80%	179	87%	148	95%	0	0%	74%	2%	2%	100%	20%
Punjab	91%	76%	5091	91%	5408	97%	3945	87%	2419	26%	55%	1%	1%	43%	64%
Rajasthan	92%	78%	12044	79%	13523	89%	10171	88%	3776	14%	16%	1%	0%	29%	43%
Sikkim	88%	70%	123	84%	131	90%	116	95%	93	30%	1%	0%	0%		
Tamil Nadu	91%	70%	8411	82%	9971	97%	6989	83%	5585	27%	88%	8%	7%	83%	58%
Tripura	90%	73%	374	81%	450	97%	305	84%	358	51%	13%	2%	0%	67%	100%
Uttar Pradesh	92%	79%	37957	91%	41566	99%	27432	87%	47530	70%	5%	2%	0%	31%	32%
Uttarakhand	90%	77%	1696	88%	1887	97%	1254	83%	1822	53%	32%	1%	0%		
West Bengal	90%	66%	11289	81%	12358	89%	9650	83%	6208	26%	43%	3%	1%	60%	54%
Grand Total	**91%**	**73%**	**178984**	**87%**	**197112**	**96%**	**135786**	**84%**	**174770**	**47%**	**39%**	**7%**	**3%**	**90%**	**55%**

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 the Hon'ble Minister for Heath and Family Welfare with State Health Ministers and Health Secretaries on 12th-13th January 2011 at Hyderabad. A Hyderabad Declaration was issued which contained 'achieving Universal Access and complete coverage for DOTS plus services' as its major objectives towards achieving control of Tuberculosis. The Health Ministers were conveyed the importance of achieving 'Universal access for TB care' at the earliest, emphasizing on the fact that no patient in the country suffering form Tuberculosis should be devoid of the best available diagnostic facility and standardized treatment.

Progress in Supervision, Monitoring and Training

Performance Indicators published in quarterly as well as annual status reports have been revised through a national consultative process. The Supervision and Monitoring Strategy for the programme is being revised, keeping in view the objective of achieving Universal Access. 17 state internal evaluations were conducted in first quarter 2011.

Progress in accreditation of Intermediate Reference Laboratories (IRL)

RNTCP has accredited 27 Solid Culture and DST laboratories in the country which include four National reference laboratories, 15 Intermediate Reference laboratories and eight laboratories from other sectors like Medical Colleges, NGOs and Private sectors, the other four laboratories are in the final stages of accreditation. The Line Probe Assay (to detect Isoniazid and Rifampicin resistance) has been introduced in the programme and three IRLs and one NRL have been accredited to deliver the services, several other laboratories are in different stages of accreditation.

Progress in the DOTS- Plus services for MDR-TB cases

DOTS Plus services for management of MDR TB are now available in 150 districts covering a population of 331.5 million in 15 states. Till date, a total of around 4217 MDR-TB patients are on treatment in these states. Other states are in various stages of preparatory activities for rolling out DOTS-Plus services.

Progress in TB HIV Collaborative Activities

Scale-up of Joint TB/HIV collaborative activities has been hailed by numerous external review missions as one of the success stories of RNTCP and continues to progress impressively. National Technical Working Group which met on 23rd April 2011 has decided to roll out Intensified TB/HIV package of service to the remaining six states and UTs; thus achieving nationwide coverage by the end of 2011. However, the performance has been uneven and varied across the states especially in linking HIV-infected TB patients to ART and this requires intensive supervision and monitoring. As we plan for the next Five Year Plan (2012-17), RNTCP and NACP are preparing a joint national strategic plan with inputs from all stakeholders to achieve universal access to TB/HIV care – All TB patients to be tested for HIV and all HIV-infected TB patients to be receiving CPT and ART.

ABSTRACTS

Concordance of a positive tuberculin skin test and an interferon gamma release assay in bacille Calmette-Guerin vaccinated persons

C.S. Mahan, D. F. Johnson, T.C. Curley, F. van der Kuyp. *Int J Tuberc Lung Dis* 2011; **15(2)**: 174-8.

False-positive tuberculin skin test (TST) results due to prior bacille Calmette-Guerin (BCG) vaccination may lead to unnecessary treatment of presumed latent tuberculosis infection (LTBI). Recently approved interferon-gamma release assays (IGRAs) are more specific for LTBI in this group. A total of 316 BCG-vaccinated foreign-born individuals with a positive TST had a commercially available IGRA (QuantiFERON®-TB Gold In-Tube) performed as part of a two-step procedure to determine the need for isoniazid therapy. Baseline demographic information and TST size were recorded and analyzed for characteristics associated with an increased likelihood of having a positive IGRA. Increasing age, male sex, origin from a country with a high prevalence of tuberculosis (TB), shorter time since arrival in the United States, and increasing TST size were all independently associated with a positive IGRA. Patient characteristics and TST size can help determine those at highest risk for LTBI. A two-step procedure for LTBI screening should be considered for foreign-born persons with prior BCG vaccination and a positive TST.

The sensitivity of interferon-gamma release assays is not compromised in tuberculosis patients with diabetes

M. C. Walsh, A. J. Camerlin, R. Miles, P. Pino, P. Martinez, F. Mora-Guzman, J. G. Crespo-Solis, S. P. Fisher-Hoch, J. B. McCormick and B. I. Restrepo. *Int J Tuberc Lung Dis* 2011; **15(2)**: 179-184.

The sensitivity of the interferon-gamma release assays (IGRAs) in the detection of *Mycobacterium tuberculosis* infection or disease may be affected by immune dysregulation in diabetes. As millions of type 2 diabetes patients are at risk for tuberculosis (TB) worldwide, it is important to determine if the sensitivity of IGRAs is compromised in this vulnerable population. The sensitivity of the IGRAs QuantiFERON®-TB Gold (QFT-G) and T-SPOT®. TB was evaluated among specimens from newly diagnosed adults with microbiologically confirmed TB with and without diabetes. We also evaluated the association between QFT-G results and diabetes-associated conditions (dyslipidemia, obesity). QFT-G sensitivity was 70% among TB patients. Patients with diabetes, chronic hyperglycemia or overweight/obesity were more than twice as likely to have positive test results in multivariate models *($P <$* 0.05). Low high-density lipoprotein cholesterol or high triglycerides were not associated with assay results. In a separate group of TB patients *(n=43)*, *T-SPOT. TB* was 93% sensitive, with similar performance in patients with and without diabetes. IGRA sensitivity is not compromised by diabetes in TB patients. Accordingly, IGRAs may also be suitable for diagnosing TB infection in diabetes patients, which is required to assess TB risk.

Missed opportunities for tuberculosis diagnosis

S. L. Bailey, M. H. Roper, M. Huayta, N. Trejos, V. Lopez Alarcon and D. A. J. Moore. *Int J Tuberc Lung Dis* 2011; **15(2)**: 205-10.

In high tuberculosis (TB) burden, resource-poor countries, sputum smear microscopy remains the mainstay of diagnosis. The low sensitivity of this test means that patients with smear-negative but culture-positive TB pass undetected through the health care system. Such clinical episodes are missed opportunities for diagnosis and interruption of transmission, which might be averted through the application of more sensitive diagnostic tests. The objective was to estimate the proportion of incident TB cases that might have been detected earlier than the actual date of diagnosis if a test more sensitive than smear microscopy had been used at an earlier

presentation episode. It was a retrospective cohort study in urban Peru, investigating health care facility interactions for symptoms suggestive of TB prior to TB diagnosis through patient interviews and a review of clinical records. Of 212 participants enrolled, 58% had one or more clinical interactions prior to their diagnostic episode. Of those with a prior episode, the median number of episodes was three. The median delay to diagnosis from first presentation was 26 days. There are clear missed opportunities for earlier TB diagnosis, delaying treatment initiation and continued spread of *Mycobacterium tuberculosis* to the community. The implementation of sensitive diagnostic tests appropriate to resource-poor settings should be given high priority.

A **cost-benefit analysis of scaling up tuberculosis control in India**

M. Goodchild, S. Sahu, F. Wares, P. Dewan, R. S. Shukla, L. S. Chauhan and K. Floyd. *Int J Tuberc Lung Dis* 2011; **15(3)**: 358-62.

The objective was to measure the economic costs and benefits of scaling up tuberculosis (TB) control under the Revised National Tuberculosis Control Programme (RNTCP) in India. The design was modelling based on country-level programme and epidemiological data from 1997 to 2006. The scale-up of TB control in India has resulted in a total health benefit of 29.2 million disability-adjusted life years (DALYs), including 1.3 million deaths averted. In 2006, the burden of TB measured in terms of DALYs lost would have been 1.8 times higher in the absence of the programme. The total gain in economic well-being from TB control is estimated at US$88.1 billion over the 1997-2006 10-year period. Total public expenditure on TB control over this period amounted to US$768 million, with the RNTCP accounting for US$299 million and other health sector costs accounting for US$469 million. The cost of TB control averaged just US$26 per DALY gained over 1997-2006 and generated a return of US$115 per dollar spent. The scale-up of TB control has been a very cost-effective strategy for improving the health status of India's population, while the return on investment has been exceptional from a societal perspective.

Enhanced tuberculosis identification through 1-month follow-up of smear-negative tuberculosis suspects

A. Porskrog, M. Bjerregaard-Andersen, I. Oliveira, L. C. Joaquim, C. Camara, P. L. Andersen, P. Rabna, P. Aaby and C. Wejse. *Int J Tuberc Lung Dis* 2011; **15(4)**: 459-64.

It was a Bandim Health Project, Bissau, Guinea-Bissau with the objective of conducting tuberculosis (TB) screening among former TB suspects in whom TB had been ruled out on initial consultation and therefore assumed to be TB-negative (aTBneg). In a cohort follow-up study, 'aTBneg suspects' were screened for symptoms from one month after the initial negative sputum smear examination. Symptomatic individuals were referred for clinical re-examination and human immunodeficiency virus (HIV) testing. Among 428 TB suspects presenting over a 10-month period in 2007, 80% (343) were smear-negative. Of these, 21 were subsequently diagnosed with smear- negative TB. Of the remaining 322 aTBneg patients, 212 were followed up and symptoms were examined $\geq$ 1 month after initial examination. Among followed up patients, 89 (42%) were still symptomatic: five were diagnosed with TB on the basis of repeated sputum smears and chest X-ray. Of 44 symptomatic patients, 39% *(n* = 17) were HIV-infected. Thirteen (4%) of the 322 aTBneg suspects died before follow-up. A large proportion of aTBneg patients remained symptomatic after one month. Several TB cases had initially not been diagnosed, and HIV infection was highly prevalent. aTBneg suspects have a high mortality rate and need increased attention from both TB and HIV programmes.

Rapid detection of multidrug-resistant *Mycobacterium tuberculosis* by multiplex allele-specific polymerase chain reaction

B. R. Imperiale, A. A. Cataldi and N. S. Morcillo. *Int J Tuberc Lung Dis* 2011; **15(4)**: 496-501.

The study was done at the Dr. Cetrangolo Hospital, Buenos Aires Province, Argentina with the objective of evaluating a multiplex allele-specific polymerase chain reaction (MAS-PCR) to detect

multi-drug resistant tuberculosis (MDR-TB) clinical isolates and to describe the main mutations conferring resistance to isoniazid (INH) and rifampicin (RMP). Drug-resistant *Mycobacterium tuberculosis* clinical isolates were tested to detect mutations using MAS-PCR. The genes involved were *kat*G, *inh*A promoter and *rpo*B. Among 193 clinical isolates included in the study, 52.6% of the INH-resistant isolates presented a mutation in the *kat*G (315) gene, 28.1% in the *inh*AP (-15) and 3.0% in both. For the *rpo*B gene, 60% of the RMP-resistant isolates showed a mutation in codon 531,17.5% in 526 and 2.5% in 516. Results were compared with those obtained by sequencing, and 100% concordance was obtained for the detection of the mutation in *kat*G (315), 94.1% for *inh*AP (-15), and 97.8% for *rpo*B. The global concordance between both methods was 98%. The MAS-PCR system allowed the simultaneous and rapid detection of approximately 80.0% of the drug-resistant clinical isolates. This method could be used as a rapid and simple screening tool to detect drug-resistant TB in clinical practice.

Molecular genotyping of *Mycobacterium tuberculosis* in Xi'an, China, using MIRU-VNTR typing

A. Zhou, M. Nawaz, X. Xue, P. C. Karakousis, Y. Yao and J. Xu. *Int J Tuberc Lung Dis* 2011; **15(4)**: 517-22.

The molecular genotyping of *Mycobacterium tuberculosis* is expected to lead to a better understanding of *M. tuberculosis* transmission in Xi'an, one of the largest cities in Western China. The objective was to characterize the population of *M. tuberculosis* strains circulating in Xi'an and to evaluate the feasibility of the mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR) typing method. A total of 195 *M. tuberculosis* clinical isolates from Xi'an were genotyped using spoligotyping and MIRU-VNTR. Thirty-two different genotypes were identified by spoligotyping, with the Beijing family identified as the largest lineage (84.6%), followed by the Tl (3.08%), T2 (1.54%), H3 (1.03%) and U (1.03%) families. The Hunter-Gaston discriminatory index (HGDI) of spoligotyping is low (HGDI = 0.392). In contrast, 15-locus MIRU-VNTR shows a higher discriminatory power (HGDI = 0.998) than 12-locus MIRU-VNTR (HGDI = 0.948). By replacing the ETR C locus with the MIRU39 locus, the discriminatory power of 15-locus MIRU-VNTR is increased to 0.999. The Beijing family accounts for the vast majority of *M. tuberculosis* isolates in Xi' an, China. The modified 15-locus MIRU-VNTR showed high HGDI and can be used as a first-line genotyping method in combination with spoligotyping in routine epidemiological investigations in Xi'an, China.

Clinical application of Line Probe Assay (LiPA) for Rifampicin (RFP)-resistant gene examination in sputum from tuberculosis patients

Takayuki INAGAKI, Tetsuya YAGI, Kazuya ICHIKAWA, Taku NAKAGAWA, Makoto MORIYAMA, Kei-ichi UCHIYA, Toshiaki NIKAI, and Kenji OGAWA . *Kekkaku* 2010; 85(9): 703-09.

Preventing the spread of drug-resistant tuberculosis is a clinically important challenge. In this effort, rifampicin (RFP)-resistant gene examination by line probe assay (LiPA) was evaluated for its clinical application for rapid detection of tuberculosis. The RFP-resistant gene was examined in a total of 110 samples of sputum obtained from patients that were definitively diagnosed with pulmonary tuberculosis by auto- LiPA. The difference in detection sensitivity between the results of the smear and culture examinations was evaluated. Culture-positive samples were compared with the results of the drug susceptibility test. Smear-positive samples were LiPA positive in 69 of 73 samples (sensitivity: 94.5%), and smear-negative samples were LiPA positive in 25 of 37 samples (67.6%). More than half of the samples were LiPA positive, even those that were culture-negative or contaminated. Comparison of the 76 culture-positive samples with the results of the drug susceptibility test found that all samples were wild type among the RFP-sensitive strains. Among the eight RFP-resistant strains, six were mutation type. All samples shown to be mutation type were obtained from patients with multi-drug resistant tuberculosis. Using LiPA, the amount of smear can be used as a factor for detection of RFP-resistant genes. Detection was possible even with culture-negative and contaminated samples, allowing more rapid diagnosis of patients with multi-drug resistant tuberculosis.

Indian J Tuberc 2011; 58: 139-142

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.

SUBMISSION OF ARTICLES

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.

PREPARATION OF MANUSCRIPTS

Manuscripts should conform to the Uniform Requirements for Manuscripts submitted to the Biomedical Journals (for further details see Ann Intern Med 1997; 126: 36-47). Articles on clinical research should conform to the standards defined in the Helsinki Declaration.

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.

All submitted manuscripts should have a definite format comprising the following sections: Title page, Summary, Introduction, Material and Methods, Results, Discussion, Acknowledgements and References.

Title page

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.

Summary

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

summaries may be submitted for review articles, case reports and short communications (100 words).

Text

Heading should conform to the text of the article. Normally only two categories of heading are used. Major headings shou1d be in capital letters and minor in upper lower case letters at the left-hand margin. The sub-titles should not be numbered in figures or alphabetically

The text should be written as lucidly as possible.

Numerals should be spelt out from one to nine (except measurement) and when beginning a sentence.

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.
Material and Methods: used in the study.
Results: Presented in logical sequence in the text, with tables and illustrations. All the results of the tables should not be repeated in the text; only important results should be emphasized.
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.

In case reports, the sections on *"Material and Methods"* and *"Results"* are replaced by the section *"Clinical Record',* and all other sections are appropriately shortened.

2. Other papers can be sub-divided, as the authors desire: the use of headings enhances readability.

References

References cited in the text and given at the end of the manuscript should conform to the Vancouver style. The authenticity of the references is the responsibility of the author. They must be numbered in the order in which they are cited in the text, and should be numbered in Arabic numerals in superscript. References that are cited more than once should retain the same number for each citation. The truly scientifically acceptable references are those of publications that can be consulted. Permission from the source(s) of information for citing their work must be obtained beforehand. All the numbered references in the text should be typed out in detail at the end of the manuscript, in the same numerical order as they appear in the text.

Journal: References to an article in a periodical should include the authors' names (list all authors when six or fewer, when there are more, list only the first three authors and add "et al"), the full title of the article, the name of the cited journal in its usual abbreviated form according to the *Index Medicus,* year of publication, tome or volume number, first and last page numbers in full:

e.g. Jain NK, Chopra KK, Prasad G. Initial and Acquired drug resistance to Isoniazid and Rifampicin and its implications for treatment. *Indian J Tuberc* 2002; **39**: 121-24.

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.

Chapter in a book: Reference to a chapter in a book should include the authors' names, the title of the chapter with the word "In" preceding the reference of the work:

e.g. Fraser RS, Muller NL, Colman N, Pare PD. Upper airway obstruction. *In:* Fraser

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.

Reference to electronic material: If references are made to electronically published material, as much of the information as for other reference sources should be provided, the html address and the date last accessed.

Personal communication: References to personal communications should be given in the text with the name of the individual cited and with his/her consent.

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.

Tables

Tables should be referred to consecutively in the text, placed after the list of references on separate sheets of paper, and should be numbered in Arabic numerals which are used for reference in the text. A short descriptive title should appear above the table, each column should have a short or abbreviated title. All abbreviations and necessary explanatory notes should be given below the table. The number of tables should be kept to a basic minimum to explain the most significant results.

Figures

Figures should be referred to consecutively in the text, placed after the list of references on separate sheets of paper, and should be numbered in Arabic numerals which are used for reference in the text. A short descriptive title should appear above the figure. Figures can be inserted into the word document for submission or uploaded separately as image files (.jpg, .gif, or .tif). If this is not possible, good quality (camera ready) prints of the figures must be provided.

Line drawings (curves, diagrams, histograms) should be provided in black and white. For optimal clarity, avoid shading.

Half-tone figures should be clear and highly contrasted in black and white. Photo- micrographs should have internal scale where appropriate. X-ray films should be careful1y made to bring out the details to be illustrated with an overlay indicating the area of importance.

Illustration: Legends for photographs should be typed separately with appropriate indication regarding the photograph to which a legend pertains. Photographs (black and white prints) should be clear, glossy and unmounted. Facilities for printing photographs in four colours as illustrations in case reports are available. Contributors are requested to preferably send colour photographs of their clinical material. Each photograph should carry, on its reverse, the title of the paper, and an arrow indicating the top edge of the photograph in pencil. It should be put in an envelope and properly labelled on the outside and attached to the article.

Patient confidentiality: Where illustrations show recognisable individuals, consent must be obtained for publication. If not essential to the illustration, authors should indicate where it can be cropped, or mask the eyes.

Permission to reproduce illustrations or *tables* should be obtained from the original publishers and authors, and submitted with the article by email or fax. They should be acknowledged in the legends as follows:

"Reproduced with the kind permission of (publishers) from (reference)"

Abbreviations and units

Avoid abbreviations in the title or summary. All abbreviations or acronyms used in the text must be defined at the first mention, and should be kept to a minimum. Symbols and units of measure must

conform to recognized scientific use i.e. SI units.

LENGTH OF TEXT

Editorial text can be up to 500 words with five references

Review articles are from those especially requested persons, who have acknowledged competence in given subjects. Text can be up to 4500 words, a structured or unstructured summary of maximum 250 words, 10 tables/figures and 50 references. **Leading articles** are by those who have expertise in selected aspect of a subject.

Original articles deal with planned studies that have been duly completed and convey definite conclusions from the data presented in the text. Text can be up to 2500 words, a structured summary of maximum 250 words, seven tables/figures and 35 references. Preliminary communications from research still in progress could be submitted exceptionally, if the topic is important and the interim results could be of interest.

Short communications can be of a text up to 1000 words, a summary of 100 words, two tables/figures and 10 references.

Case reports present problems of unusual clinical interest which have been systematically and fully investigated and where a firm diagnosis has been established with reasonable certainty, or the result of therapeutic management is of significance. Text can be up to 1000 words, a summary of 100 words, two tables/ figures and 10 references.

Workers in the field of Tuberculosis and Respiratory Diseases are invited to contribute to the **Radiology Forum** by submitting brief reports of patients with interesting clinical and radiological features for publication. These will be published, provided that:

(a) the condition is of clinical and radiological interest;
(b) photographs (10 cm x 8 cm) are of suitable quality for printing;
(c) the diagnosis in each case has been confirmed;
(d) the chest radiograph is accompanied by brief clinical account, not exceeding 500 words, and five references

Forum, in the form of letters to the Editor, provides a platform to readers for expressing their opinions and is a channel of communication with the journal and its readers. It could be used for making suggestions, scientific critique on published articles or for reaching independent conclusions, for asking questions on subjects covered by the journal and for providing supplementary information, either confirming or contradicting the conclusions reached in the article. Such letters can be up to a text of 1000 words with two tables/figures and 10 references. Only the most important agreements, disagreements/suggestions may be chosen for commenting. It is usual to send a copy of such letters to the authors for obtaining a response, if any, after editorial changes. The response, similarly, has to be brief and relevant.

Correspondence can be up to 500 words without tables or figures and five references.

IJT has been indexed in MEDLINE of National Library of Medicine, USA

The journal is also available online at the website http://medind.nic.in.