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Indian Journal of Tuberculosis

Vol. 57 **New Delhi, April, 2010** **No. 2**

Editorial

EXPANDING DOTS – NEW STRATEGIES FOR TB CONTROL?

[Indian J Tuberc 2010; 57:63-66]

Tuberculosis continues to be a major public health problem in the world, particularly in the developing countries. The updated WHO report reveals that about 9.4 million (8.9–9.9 million) new TB cases occurred in 2008 (3.6 million, of whom are women) including 1.4 million cases among people living with HIV. The prevalence of the disease was about 11.1 million (9.6–13.3 million prevalent cases). There were about 1.3 million (1.1–1.7 million) deaths from TB among HIV-negative people and an additional 0.52 million (0.45–0.62 million) TB deaths among HIV-positive people[1]. India is the highest TB burden country in the world, accounting for 21% of the global incidence and 2/3rd of the cases in South East Asia. In the year 2008, the incidence of tuberculosis was reported to be 1.982 million (1.586-2.379 million) with prevalence of 2.186 million (1.044 – 3.739 million) with mortality due to TB being 2, 76, 512. The percentage of HIV positivity in that year was 6.7% with a range of 5.5 – 7.9%.[1-3].

The WHO declared TB a global emergency in 1993 realizing its growing importance as public health problem. It developed the DOTS strategy (Directly Observed Treatment, Short Course) in 1994 as the new frame work for effective TB control[4-7] with five components. The strategy has been adopted in many countries with flexibility and adaptation to the existing needs of the community[8,9].

The global targets for TB control, adopted by the World Health Assembly, are to cure 85% of the newly detected sputum smear positive TB cases and to detect 70% of the estimated incidence of sputum smear-positive TB case[10]. Although many countries have achieved this target, the case detection rate was 63% globally in 2007 through the DOTS programmes and the same for all cases was 56%. 36 million people with TB are cured and up to 8 million lives are saved through 15 years of DOTS programmes, confirming that DOTS as the most cost effective approach in the fight against tuberculosis but millions still unable to access high quality care[1,11]. While the global incidence of TB appears to have been declining slowly since 2004, and treatment success was as per the target in 2006, the case detection rate for sputum smear-positive TB is stagnating at 64% in 2007[12].

Many countries at the global level including India has achieved the initial set target of 70% of case detection rate and 85% cure rate[13]. Is this strategy enough to control TB? In a simple mathematical calculation, out of 100 cases of TB, the current programme is detecting 70 cases and with a success rate of treatment under DOTS being 85%, in fact out of these 100 patients, only 59.5 patients are actually being cured. This means that a large chunk is still not being covered/cured/treated. 70% of case detection still leaves behind a gap of 30% of cases yet to be detected. The issues of HIV, drug resistant tuberculosis like MDR and XDR-TB complicated matters further. For such a large programme, huge amount of funding is required. The drugs are still old and in the recent past there is no new drug discovery. Vaccines are still a distant dream. The Stop TB strategy has adopted seven key areas and the Stop TB Partnership's seven key approaches are: DOTS expansion; DOTS-Plus for multidrug resistant TB; TB/HIV Collaborative activities; Newer TB diagnostics; Discovery of new TB drugs; New TB vaccines, Advocacy and of course adequate funding. All these factors need to be taken into account before we dream of a TB-free

world or to achieve the million development goals even if, there are indications that there is some progress towards this.

Then how can we achieve such goals? Besides maintaining and sustaining the current achievements, quality DOTS expansion has to be made which is perhaps the key factor. To increase the case detection and to have wider access of TB services to each and every body in the community, we need to develop newer strategies. An action framework for higher and earlier TB case detection has been proposed by the DOTS Expansion Working Group of the Stop TB Partnership. Several possible reasons for low case detection rate and delay treatment have been identified. They include poor understanding of TB and its symptoms in the general population, poor knowledge where to seek care, poor health service infrastructure with limited out reach, barriers to access, poor diagnostic quality, limited human resource for health, poor TB knowledge amongst health providers, perverse incentive systems for providers that foster us of inappropriate medical technologies, poor coordination of health services and poor information systems including notification and referral routines. These factors may be different in different settings and they need to be identified by analyzing the gaps and barriers for early case detection. Some of the priority actions may include intensifying the case finding strategies in health care facilities. The diagnostic algorithm should go beyond the current passive case finding strategies i.e. unexplained cough for two weeks or more. Any cough of any duration may be used as a screening indication in a high burden setting. Although this will maximize sensitivity, more tests will be performed on people who do not have TB and will unnecessarily burden the resources. Fluorescent microscopy using LED microscopes will improve the case identification. The earlier mass radiology may be used in selective cases, particularly those who are sputum negative but having a high index of suspicion. Besides contact investigation other active case finding strategies do exist. The mass radiography screening as was done earlier has been discouraged by the WHO expert committee on tuberculosis in the 1960's and 70's[14]. However, there are several alternatives to mass screening, which are more targeted, less resource demanding and more cost effective. This includes screening of risk groups with high TB exposure such as certain health care workers, prisoners, refugees, drug addicts, homeless people, slum dwellers and other identified high risk population. Such screening may be combined with communication strategies to encourage people to approach health facilities if they have TB symptoms.

Practical approach to lung health (PAL) is a newer initiative wherein respiratory conditions are usually diagnosed in the primary health care settings. Patients with persistent respiratory symptoms including TB suspects are often mismanaged in these settings. PAL approach can screen for TB among respiratory patients who meet the definition of TB suspects and thus this is a recommended approach to maximize case detection especially for middle income countries. Further improvement can be made by improving diagnosis of extra-pulmonary TB and TB in children. It is emphasized that there is a need to screen all people with HIV for TB regardless of symptoms. The diagnostic algorithm is different for these cases mainly because of the need to treat them early which is more critical among people with HIV. Both the programmes should work together to improve case detection and early treatment. The house-hold contacts in a case of tuberculosis need to be screened thoroughly. Other clinical risk groups that can be brought under the umbrellas of screening include smokers, diabetes mellitus, malnutrition, alcoholism, immunosuppressive states like cancer, steroid use, use of other immunosuppressive drugs, certain occupations like silicosis etc.[15-19]. In addition, people with previous tuberculosis are at higher risk than the general population to develop active TB and the case finding's yield may be higher when these patients are screened for active disease. Certain cases of tuberculosis can present without any respiratory symptoms but only with systemic features like pyrexia of unknown origin, weight loss, anorexia, vague ill health, etc. that may need special attention for screening for tuberculosis. However, this syndromic approach should be made very cautiously to avoid over-diagnosis. The programme should see that there are minimum

access barriers, especially for the poor and the vulnerable. In fact, many national programmes have decentralized service delivery to ensure access to all patients including those in remote areas or difficult areas. However, there may be important gaps in such geographical coverage. What is to be done in a case of natural disaster or in terrorist/extremist affected areas? It is well recognized that the poorest of poor, those living in difficult and remote rural areas, in conflict zones and in urban slums that lack basic health care facilities, often have poor access to quality services. Certain groups like that the disempowered, uneducated or poorly educated individual, marginalized section of the society and illegal migrants will have great difficulties both accessing the care and fully availing these available services even if they can reach the appropriate facility. One such important group in settings of developing countries is the migratory population and the destitute.

Gender bias is an important issue in many social settings where women seem to face special access barriers like stigma and lack of financial resources. All health care providers need to be engaged. As discussed above, only about 60% of the TB patients are brought under the cover of the national prgrammes. The remaining persons either avail treatment which is not under direct supervision and without recording or reporting of the treatment outcome. This is because of different health care facilities which may be diverse in particular settings. All these practitioners outside the DOTS programme should be brought under the programme and some system of notification, either legal or voluntary, should be enforced in the society. A variable proportion of patients approach private provider first that include both the poor and the rich. Guidelines have been developed for the engagement of all such health providers through the Public-Private Mix (PPM) approach. This approach should be implemented more vigorously with greater efforts. Health communication and social mobilization is one of the key areas for case detection and patient's access to the programme that need to be strengthened further. A powerful way to increase the utilization is to ensure that high quality accessible and affordable services are in place. Community participation as well as the rights of the patient for the diagnosis and treatment need to be enforced and emphasized. General health system strengthening is another strong method like the integration/coordination of the programme with National Rural Health Mission (NRHM).

TB control will not be possible without attending to MDR and XDR-TB (through prevention of drug resistance[20] through sustained high-quality DOTS implementation, improving laboratory capacity, effective treatment of patients through DOTS Plus services, promoting rational use of anti-TB drugs in the country and implementing infection control measures) and TB-HIV issues. Arrangement of funding and its judicious use in the era of economic slow down is another important key area that needs to be attended to.

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REFERENCES

1. Global tuberculosis control: a short update to the 2009 report. "WHO/HTM/TB/2009.426".
2. Minutes of the Expert committee meeting to estimate TB burden in India. March 2005. Directorate of Health and Family Welfare, Central TB Division, Government of India, 2005. Available at http://www.tbcindia.org.

3. Gopi PG, Subramani R, Santha T, Chandrasekaran V, Kolappan C, Selvakumar N, et al. Estimation of burden of tuberculosis in India for the year 2000. *Indian J Med Res* 2005; **122**: 243-8.
4. Behera D. TB Control: role of DOTS. *Expert Rev Resp Med* 2009; **3**: 557-60.
5. Raviglione MC, Pio A. Evolution of WHO policies for tuberculosis control, 1948-2001.*Lancet* 2002; **359**: 775-80.
6. Garner P, Volmink J. Directly observed treatment for tuberculosis. Less faith, more science would be helpful. *BMJ* 2003; **327**: 823-4.
7. Dye C, Garnett GP, Sleeman K, Williams BG. Prospects for worldwide tuberculosis control under the WHO DOTS strategy. *Lancet* 1998; **352**:1886-91.
8. World Health Organization. Community contribution to TB care: practice and policy. WHO Stop TB Department, Geneva, 2003. (WHO/CDS/TB/2003.312).
9. Maher D, Uplekar M, Blanc L, Ravglione M. Treatment of tuberculosis, Concordance is a key step. *BMJ* 2003; **327**: 822-3.
10. Matthys F, Van der Stuyft P, Van Deun A. Universal tuberculosis control targets: not so smart. *Int J Tuberc Lung Dis* 2009; **13**: 923-4.
11. World Health Organization. Global Tuberculosis Control 2009. Epidemiology, Strategy, Financing. WHO/HTM/TB/2009.411. Geneva, Switzerland: WHO, 2009.
12. Resolution WHA44.8 of the Forty-forty-fourth World Health Assembly, Geneva, World Health Organization, 1991 (WHA44/1991/REC/1), and Resolution WHA46.36 of the Forty-sixth World Health Assembly, Geneva, World Health Organization. 1993.
13. TB India 2008. RNTCP Status Report.
14. WHO. WHO Expert Committee on Tuberculosis. Ninth report. WHO Technical Series, No 552.Geneva: World Health Organization, 1974.
15. Slama K, Chiang CY, Enarson D, Hassmiller K, Fanning A, Gupta P, Ray C. Tobacco and tuberculosis: a qualitative systematic review and meta analysis. *Int J Tuberc Lung Dis* 2007; **11**: 1049-61.
16. Stevenson CR, Critchley JA, Forouhi NG, Roglic G, Williams BG, Dye C, Unwin NC. Diabetes and the risk of tuberculosis: a neglected threat to public health? *Chronic Illness* 2007; **3**: 228-45.
17. Cegielski P, McMurray DN. The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals. *Int J Tuberc Lung Dis* 2004; **8**: 286-98.
18. Lönnroth K, Williams BG, Stadlin S, Jaramillo E, Dye C, Raviglione M. Alcohol use as risk factor for tuberculosis disease - a systematic review. *BMC Public Health* 2008; **8**: 289.
19. Rieder H. Epidemiologic basis of tuberculosis control. Paris: International Union Against Tuberculosis and Lung Disease, 1999.
20. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response. WHO/HTM/TB/2010.3.

Original Article

DETECTION OF CIRCULATING FREE AND IMMUNE-COMPLEXED ANTIGEN IN PULMONARY TUBERCULOSIS USING COCKTAIL OF ANTIBODIES TO *MYCOBACTERIUM TUBERCULOSIS* EXCRETORY SECRETORY ANTIGENS BY PEROXIDASE ENZYME IMMUNOASSAY*

Anindita Majumdar[1], Pranita D. Kamble[2] and B.C. Harinath[3]

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Summary

Background: Decreased sensitivity has been a limiting factor of antigen assay for detection of tuberculosis. Assay of more than one antigen may improve sensitivity of an assay.

Aim: To develop a simple, rapid and less-expensive serodiagnostic method compared to culture method for Pulmonary Tuberculosis.

Method: A cocktail of affinity purified antibodies against *Mycobacterium tuberculosis* H_{37}Ra antigens (SEVA TB ES-31, ES-43 and EST-6) was explored for detection of circulating free and Immune-Complexed (IC) cocktail antigen by microtitre plate Peroxidase sandwich ELISA. The assay was evaluated in 27 clinical sera of sputum acid fast bacilli (AFB) positive and 10 AFB negative but anti-tuberculosis therapy responded pulmonary tuberculosis patients and 20 normal sera as controls.

Results: Assay of cocktail antigen showed marginal improvement in sensitivity compared to assay of ES-31 antigen alone. The assay for circulating free cocktail antigen showed a sensitivity of 77.7% for AFB positive cases and 70% for AFB negative cases compared to assay of ES-31antigen with sensitivity of 74% and 70% respectively. The assay for IC-cocktail antigen showed sensitivity of 77.7% for AFB positive and 80% for AFB negative cases compared to assay of IC-ES-31 antigen with sensitivity of 77% and 70% respectively. Specificity of antigen assay was found to be 90%. Detection of IC-antigen as adjunct assay improved the sensitivity of detection in AFB-ve but ATT responded cases. Peroxidase enzyme immunoassay of cocktail antigen showed a sensitivity of detection of 0.25 µg/ ml and levels of free and IC cocktail antigens were 1.70 ± 1.04 and 1.13 ± 0.047 µg/ ml in AFB positive patients' sera.

Conclusions: Peroxidase enzyme immunoassay for circulating antigen was found to be a useful serodiagnostic assay and in particular in AFB –ve cases responding to ATT. ***[Indian J Tuberc 2010; 57: 67-74]***

***Key words*:** Mycobacterial ES Cocktail antigen, Pulmonary tuberculosis, Peroxidase ELISA

INTRODUCTION

Tuberculosis (TB) control has been a challenging problem for the medical personnel due to lack of precise diagnosis and long duration of treatment. As per global tuberculosis control - a short update to the 2009 report; World health organization estimated 9.4 million incident cases (equivalent to 139 cases per 100 000 population) of TB globally in 2008. Most of the estimated number of cases in 2008 occurred in Asia (55%) and Africa (30%). But the number of notified cases of TB in 2008 was 5.7 million, equivalent to 55–67% of all incident cases. India and China alone account for an estimated 35% of TB cases worldwide. Among these new cases, around 15% were HIV-positive[1].

The control of TB depends on early detection of cases and effective treatment[2, 3]. Diagnosis of TB using acid-fast staining of sputum smear and standard culture is considered as the 'gold standard', but sputum smear examination has shown a

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sensitivity of 40–75%,[4] and clinicians either have to treat based on clinical judgement or wait for culture results, which may take up to six weeks[5,6]. Empirical treatment increases public health expenditure and the risk of drug side-effects that may be fatal[7]. Nucleic acid amplification test seems to help in the diagnosis of TB[8]. However, this technique is expensive and requires expertise and special equipments. Hence, there is critical need for improved and handy diagnostic methods that are simple, rapid, inexpensive, reliable and suitable for use in the developing world.

Serological tests are simple to use and rapid for pulmonary tuberculosis and also useful for detecting extra-pulmonary TB and in children or uncooperative patients, among whom collection of clinical samples may be difficult. In earlier studies from our laboratory, we have shown diagnostic usefulness of *M. tb*. excretory secretory (ES) antigens ES-31, ES-41 and ES-43 in antibody detection by penicillinase ELISA (Pen-ELISA)[9-12]. Antigen EST-6 containing 38 and 41kDa proteins were also explored for antibody detection by Pen-ELISA[13]. A cocktail of ES-31, ES-41 and ES-43 antigens had shown improved sensitivity of Pen-ELISA compared to single ES-31 antigen in antibody detection in pulmonary TB (PTB)[14]. Further, a cocktail of affinity purified antibodies against ES-31, ES-43 and EST-6 antigens was explored for circulating free and IC antigen detection in TB by sandwich ELISA[15]. The usefulness of in-house developed Penicillinase ELISA using cocktail of antigens (ES-31, ES-43 and EST-6 antigens) and their immunoglobulins was also shown in a prospective study which was carried out at a tertiary care hospital located in rural area[16]. All these assays were based on penicillinase ELISA, which is sensitive but semi-quantitative and subjective assay. Microtitre plate Peroxidase sandwich ELISA for detection and quantitation of circulating free and IC ES-31 antigen was also shown to be useful in diagnosis of PTB cases[17].

In this study, mass screening suitable, user friendly microtitre plate Peroxidase enzyme immunoassay was standardized and evaluated for detection of circulating free and immunecomplexed antigen in AFB+ve sera of pulmonary tuberculosis using cocktail of antibodies to *Mycobacterium tuberculosis* excretory secretory antigens (ES-31, ES-43 and EST-6 antigens).

MATERIAL AND METHODS

Patients and controls

Sera samples from patients (n = 37) attending tertiary hospital of this Medical Institute having pulmonary TB (PTB) were utilized for standardization of Peroxidase immunoassay for the detection of cocktail antibody, circulating and IC-cocktail antigen. Clinical history, physical examination, baseline laboratory investigations [hemogram, tuberculin skin test, chest skigram, urinanalysis), microbiological (AFB smear and culture)] investigations or response to ATT were considered as the basis for confirmation of TB etiology. 27 sera belonged to AFB positive group and 10 belonged to AFB negative group, which were diagnosed clinically and responding to ATT. Sera samples from healthy individuals (n = 20) with no history of TB served as healthy controls.

Assay for circulating and IC-cocktail antigen was done in disease control sera samples (n = 20) which included samples from cases of leprosy (3), chronic obstructive airway disease (5), pleural effusion (3), pyrexia of unknown origin (1), chronic bronchitis (3), bronchial asthma (2), pneumonia (2) and bronchiectasis (1). Sera samples were stored at our centre's patient sera bank in 0.5 ml aliquots at -20^{0}C with 0.1% sodium azide until use. All cases included in this study had history of BCG vaccination. The study was done prospectively in blinded manner in which clinical diagnosis was not available to the laboratory personnel prior to the assay. In the present study, each serum sample had been assayed in duplicate.

Isolation *of M. tb*. ES-31, ES-43 and EST-6 antigens and their antibodies

ES-31 antigen was isolated from *M.tb*. H_{37}Ra ES antigen by affinity chromatography using anti ES-31 antibody coupled Sepharose-4B column

(Pharmacia Biotechnology AB, Uppsala, Sweden)[18]. Briefly, Cyanogen bromide-activated Sepharose 4B beads were coupled with purified anti ES-31 antibody. DSS antigen was passed through column and ES-31 antigen was eluted by glycine HCl buffer (0.01 mol/ L, pH2.5) and collected in Tris-HCl buffer (0.01M, pH8.6). Similarly ES-43 and EST-6 antigens were isolated by affinity chromatography using anti ES-43 or anti-EST-6 antibody coupled Sepharose-4B column. Cocktail antigen (ES-31, ES-43 and EST-6) was prepared by mixing the individual antigens in equal proportion.

M. tb. H_{37}Ra detergent soluble sonicate (DSS) antigen, was prepared from *M.tb*. H_{37}Ra bacilli. Briefly, bacilli were 5% phenol inactivated in 0.5M phosphate buffer (PBS, pH7.2) and incubated with sodium dodecyl suphate (SDS) extraction buffer. The supernatant as dialysed against 0.01M PBS, pH 7.2 and used as an antigen source[15]. Anti-DSS IgG antibodies were raised in goat by immunizing intramuscularly with 500 µg protein/ mL DSS antigen with 1 ml Freund's incomplete adjuvant on days 0, 20, 33 and 45. Immune sera were collected on days 32, 44, 57, 60 and thereafter fortnightly and anti-SDS IgG was isolated by 33% saturation with ammonium sulphate under ice, followed by diethyl aminoethyl-cellulose ion exchange column chromatography as described earlier[19]. Anti-ES-31, anti-ES-43 and anti-EST-6 antibodies were isolated from anti-DSS IgG by affinity chromatography using ES-31, ES-43 or EST-6 antigen coupled Sepharose-4B column[19]. Anti-cocktail antibody (anti-ES-31, anti-ES-43 and anti-EST-6) was prepared by mixing individual antibodies in equal proportion[18].

Peroxidase ELISA

The detection of circulating cocktail antigen (ES-31, ES-43 and EST-6) using affinity purified anti-cocktail antibody (anti-ES-31, anti-ES-43 and anti-EST-6) was performed by sandwich plate Peroxidase ELISA. The wells of ELISA plates (NUNC) were sensitized with optimally diluted concentration of anti-cocktail antibody 150 µg / 100µL/well in 0.06 M carbonate buffer pH9.6 overnight at 4^0C followed by blocking with 1% BSA for 2 hours at 37^0C. Plate was washed twice with PBS containing 0.05% Tween 20 (PBS/T) followed by addition of sera (dilution 1:50) in PBS/T for one hour at 37^0C, followed by three washes. Then the wells were exposed to 1:1000 diluted Goat anti-cocktail antibody IgG Peroxidase conjugate for 1 hour at 37^0C. The wells were washed five times with PBS/T with one minute interval. The colour was developed using TMB substrate (20X concentration) and the reaction stopped by using 50 µL stop solution (2N H_2SO_4). Then mean optical density at 450 nm was read with ELISA reader. For detecting IC antigen, serum samples were pretreated with Glycine-HCl buffer (0.1M) followed by heating at 65^0C for 15 minutes and neutralizing with 0.2M Tris HCl buffer, pH 8.6. Similarly ES-31 antigen was assayed in sera.

RESULTS

In the present study, the cocktail of affinity purified antibodies against *M. tb.* H_{37}Ra antigens (SEVA TB ES-31, ES-43 and EST-6) was explored for detection of circulating free and Immune-Complexed (IC) cocktail antigen by microtitre plate Peroxidase sandwich ELISA and compared with assay of ES-31 antigen.

The sera of healthy controls (n = 20) were screened to obtain cutoff OD (Mean + 2SD) by plate Peroxidase enzyme immunoassay, which was 0.279 and 0.296 for the circulating and IC-Cocktail antigens respectively (Figs. 1 and 2). The assay for circulating cocktail antigen showed a sensitivity of 77.7% for AFB positive cases and 70% for AFB negative cases with 90% specificity (Table1). The assay for IC-cocktail antigen showed a sensitivity of 77.7% for AFB positive cases and 80% for AFB negative cases with 90% specificity (Table1). 10% disease control cases showed reactivity for assay for circulating and IC-cocktail antigen, which was same as healthy control cases (Table 1). Figure 3 shows a standard graph with purified cocktail antigen at various concentrations (0.25, 0.1, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 ng/well) when assayed by using Sandwich Plate Peroxidase assay. The serum levels of Free Cocktail antigen are 1.70 ± 1.04 and 1.57 ± 0.87 µg/ml in AFB positive and AFB negative TB

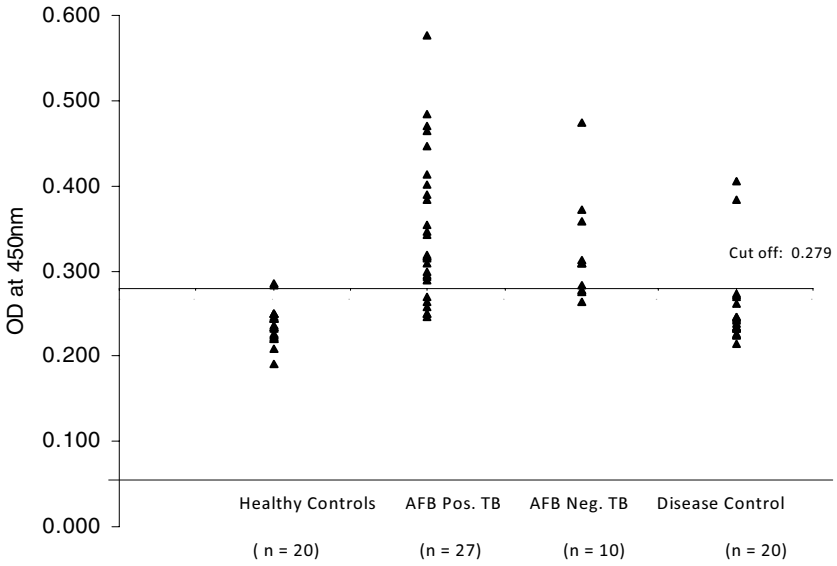

Fig. 1: Detection of Circulating Cocktail antigen in sera by Peroxidase ELISA. The mean OD_{450} obtained with sera of healthy individuals, plus 2SD, was used as the cut-off.

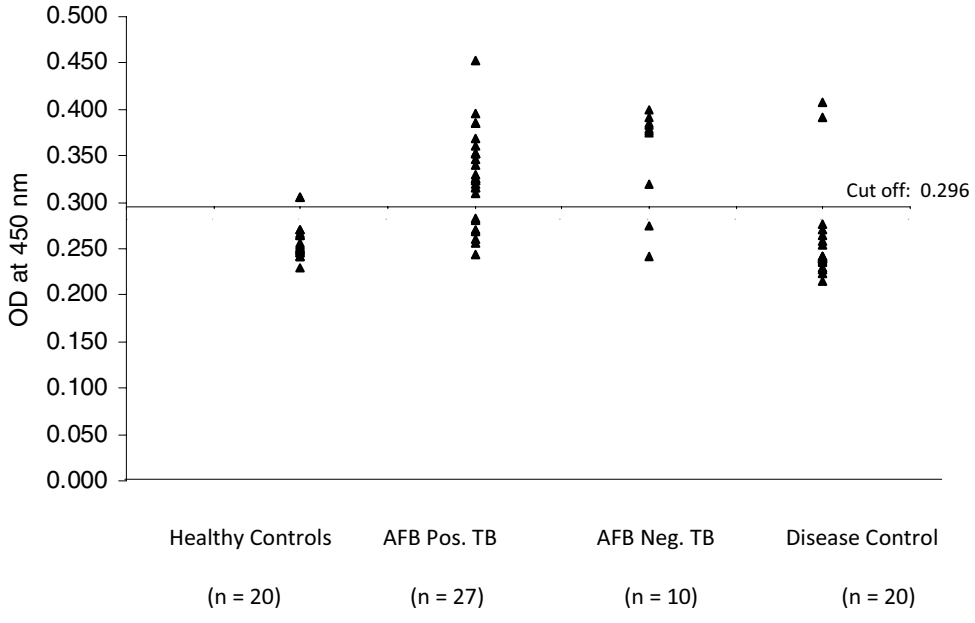

Fig. 2: Detection of IC-Cocktail antigen in sera by Peroxidase ELISA. The mean OD_{450} obtained with sera of healthy individuals, plus 2SD, was used as the cut-off.

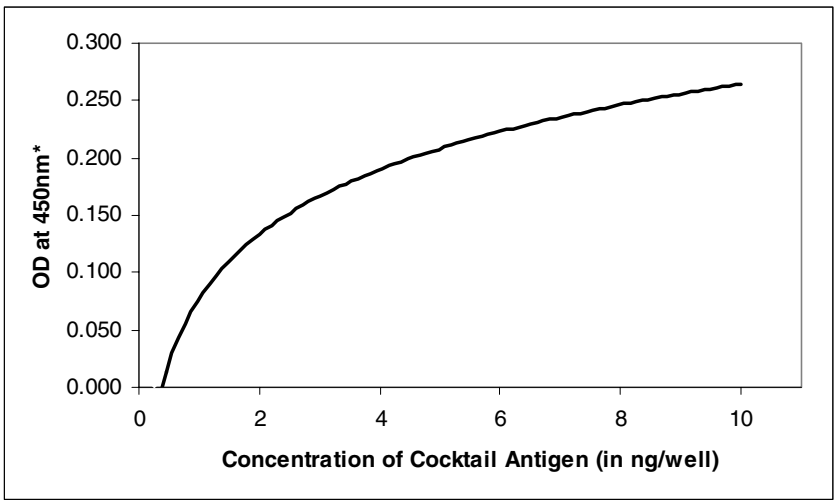

Fig. 3: Standard graph for Peroxidase assy for quantitation of Cocktail antigen +O.D. obtained after substracting mean O.D. of healthy control sera

Table 1: Detection of circulating and IC ES-31 and Cocktail antigens in sera of pulmonary tuberculosis cases

Group	No. Screened	No. (%) showing positive reaction* for			
		Free ES-31 Ag[17]	IC-ES-31 Ag	Free Cocktail Ag[17]	IC-Cocktail Ag
Pulmonary TB AFB +ve	27	20 (74%)	21 (77%)	21(77%)	21 (77%)
Pulmonary TB AFB -ve	10	7 (70%)	7 (70%)	7 (70%)	8 (80%)
Healthy control	20	2 (10%)	2 (10%)	2(10%)	2 (10%)
Disease control	20	2 (10%)	1 (5%)	2(10%)	2 (10%)
Leprosy	03	0	0	0	0
COAD	05	1	1	1	0
Pleural Effusion	03	0	0	0	1
PUO	01	0	0	0	0
Chronic bronchitis	03	1	0	1	1
Bronchial asthma	02	0	0	0	0
Pneumonia	02	0	0	0	0
Bronchiectasis	01	0	0	0	0

* sera showing positivity at 1:50 dilution.

Table 2: Levels of circulating ES-31 and cocktail antigen in Tuberculosis serum (mg/ml)

Disease status	Level of free antigen [Mean ± S.D.*]		Level of IC-antigen [Mean ± S.D.*]	
	Free ES-31 Ag[17]	Free Cocktail Ag	IC-ES-31 Ag[17]	IC-Cocktail Ag
AFB positive patients	0.71 ± 0.64	1.70 ± 1.04	0.74 ± 0.65	1.13 ± 0.47
AFB negative patients	0.82 ± 0.40	1.57 ± 0.87	0.60 ± 0.25	1.47 ± 0.33

* Standard deviation

sera respectively while the serum levels of IC-Cocktail antigen are 1.13 ± 0.47 and 1.47 ± 0.33 µg/ml of serum in AFB positive and AFB negative TB sera respectively (Table 2).

DISCUSSION

Till date, the diagnosis of TB depends on clinical findings and various laboratory tests. Although AFB smear microscopy and culture are valuable for confirmative diagnosis of tuberculosis, low bacillary load and extent of TB disease at extrapulmonary sites of the infection do make the AFB test not useful. Further, it is very difficult to obtain sputum specimen in children. Therefore immunodiagnosis seems to be ideally suited as a diagnostic method. Serodiagnostic tests like ELISA can show promise because of their ease of performance in field laboratories and cost-effectiveness.

Over a period of decade, our laboratory reported usefulness of various mycobacterial excretory secretory antigens in the diagnosis of TB by penicillinase ELISA. Cocktail of different antigens has shown to be more useful than single antigen assay[20]. Assay for detection of free circulating cocktail antigen (ES-31, ES-43 and EST-6) by penicillinase ELISA was found useful for PTB cases with 91% sensitivity and 97% specificity for sputum positive AFB positive cases[15]. Microtitre Plate Peroxidase sandwich ELISA was explored by using affinity purified anti ES-31 antibody for detection of circulating ES-31antigen in tuberculosis sera[17]. In the present study, Microtitre Plate Peroxidase ELISA was explored for detection of cocktail circulating cocktail antigen (ES-31, ES-43 and EST-6) and IC-cocktail antigens in tuberculosis sera using cocktail of antibodies.

In the present study, out of 27 AFB +ve sera, two sera did not show presence of free cocktail antigen but showed presence of IC- cocktail Ag and two sera did not show presence of IC- cocktail Ag but showed presence of free cocktail Ag. Thus, 23 sera were positive either for free or IC-Cocktail antigen. Out of 10 AFB -ve sera, two sera did not show presence of free cocktail antigen but showed presence of IC- cocktail antigen and one serum did not show presence of IC-cocktail Ag but showed presence of free cocktail Ag. Hence combination of detection of free and IC-Cocktail antigen improved the sensitivity of assay with 85% (23/27) and 90% (9/10) for AFB +ve sera and AFB -ve sera respectively. In an earlier study, the sensitivity of combined results of Free and IC-ES-31 antigen assay was 81% (22/27) and 80% (8/10) for AFB +ve sera and AFB -ve sera respectively [Table 1][17]. Thus the present assay for cocktail antigen showed marginal improvement in sensitivity compared to assay of ES-31 alone. [Table 1][17]. Antigen assay was observed to be very useful in confirming TB infection in 70% of AFB –ve but ATT responded PTB patients. Further assay of IC-cocktail antigen improved sensitivity to 80% in these cases.

Penicillinase ELISA test showed 91% sensitivity and 97% specificity for sputum positive AFB positive PTB cases for detection of free circulating cocktail antigen (ES-31, ES-43 and EST-6)[15]. In the present study, peroxidase ELISA was shown 80% sensitivity and 90% specificity for detection of PTB cases. Penicillinase ELISA using 3ìg of anti cocktail antibody for detection of cocktail antigen showed reactivity with 1:300 dilution of serum[15]; while Peroxidase ELISA using 150ìg of antibody showed reactivity with 1:50 dilution of serum. Hence Penicillinase ELISA is six fold sensitive than Peroxidase ELISA for detecting cocktail antigen in serum. The higher sensitivity in penicillinase ELISA was possibly due to high turnover number of the enzyme and sensitive colour reaction in this assay. Sauar *et al*[21] also reported the higher sensitivity of enzyme penicillinase compared to enzyme Peroxidase, alkaline phosphatase and beta galactosidase when used as labels for progesterone determination in milk by ELISA. However peroxidase enzyme immunoassay is objective and user friendly. The peroxidase ELISA assay showed a sensitivity of detection of 0.5 ìg/ ml ES-31[17] while in present study, Peroxidase assay showed a sensitivity for detection of low concentration of cocktail antigen (0.25 ìg/ ml cocktail antigen). This indicates that detection of circulating cocktail antigen may be more useful than detection of single ES-31 antigen. **It is of interest that Peroxidase ELISA could detect antigen in AFB negative but clinically diagnosed and ATT responded cases. It is of interest that AFB negative patient's, though bacillemia is low, antigen level is significant, possibly due to slow clearance of antigen in these patients. This needs further extensive study of clinically suspected AFB – ve and ATT responding TB cases.**

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REFERENCES

1. World health organization. Global tuberculosis control - a short update to the 2009 report. P- 4-5. Cited at http://www.who.int/tb/publications/global_report/2009/update/tbu_9.pdf
2. Frieden T R, Sterling T R, Munsiff S S, Watt C J, Dye C. Tuberculosis. *Lancet* 2003; **362**: 887–99.
3. Grzybowski S, Barnett G D, Styblo K. Contacts of cases of active pulmonary tuberculosis. *Bull Int Union Tuberc* 1975; **50**: 90–106
4. Kim T C, Blackman R S, Heatwole K M, Kim T, Rochester D F. Acid-fast bacilli in sputum smears of patients with pulmonary tuberculosis. Prevalence and significance of negative smears pretreatment and positive smears post-treatment. *Am Rev Respir Dis* 1984; **129**: 264–68
5. Millen SJ, Uys PW, Hargrove J, van Helden PD, Williams BG. The Effect of Diagnostic Delays on the Drop-Out Rate and the Total Delay to Diagnosis of Tuberculosis. PLoS ONE. 2008 Apr 9;3(4):e1933
6. Storla DG, Yimer S, Bjune GA: A systematic review of delay in the diagnosis and treatment of tuberculosis. *BMC Publ Heal* 2008; **8**: 15 (http://www.biomedcentral.com/1471-2458/8/15
7. Update: Fatal and severe liver injuries associated with rifampin and pyrazinamide for latent tuberculosis infection, and revisions in American Thoracic Society/ CDC recommendations—United States, 2001. *MMWR* 2001; **50**: 733–35
8. Yee Y C, Gough A, Kumarasinghe G, Lim T K. The pattern of utilisation and accuracy of a commercial nucleic acid amplification test for the rapid diagnosis of *Mycobacterium tuberculosis* in routine clinical practice. *Singapore Med J* 2002; **43**: 415–20
9. Banerjee S , Gupta S , ShendeN , Kumar S, Harinath B C. Serodiagnosis of tuberculosis using two ELISA systems. *Ind J Clin Biochem* 2003; **18**: 48-53
10. BanerjeeS , GuptaS , Kumar S, ShrikhandeA V, Reddy MVR, HarinathBC. Seroreactivityo f 31 kDa and 4lkDa mycobacteriasl ecretoryp roteinsi solatedf rom culture filtrate in extra pulmonary tuberculosis. *Indian J Pathol Microbiol* 2003: **46**: 261-64
11. Gupta S, Shende N, Kumar S, Hadnath BC. Antibody response to *M.tb*.H_{37}Ra excretory-secretory ES-43 and ES-31 antigens at different stages of pulmonary tuberculosis. *Biomed Res* 2004; **15**: 76-9
12. Bhatia AS, Gupta S, Shende N, Kumar S, Harinath BC. Serodiagnosis of Childhood Tuberculosis by ELISA. *Ind J Pediatr* 2005; **72(5):** 383-7.
13. Lodam AN, Reddy MVR, Narang P, Gupta OP, Harinath BC. Fractionation analysis and diagnostic utility of Mycobacterium tuberculosis H_{37}Ra excretory secretory antigen in pulmonary tuberculosis. *Indian J Biochem Biophys* 1996; **33**: 67-71.
14. Gupta S, Shende N, Kumar S, Harinath BC. Detection of antibodies to a cocktail of mycobacterial excretory secretory antigens in tuberculosis by ELISA and Immunoblotting. *Curr Sci* 2005; **88**: 1825-27.

15. Harinath B C, Kumar S, Roy S S, Hirudkar S, Upadhye V, Shende N. A coctail of affinity purified antibodies reactive with diagnostically useful mycobacterial antigens ES-31, ES-43 and EST-6 for detecting the presence of *Mycobacterium tuberculosis*. *Diag Microbio Inf Dis* 2006; **55:** 65-8.
16. Majumdar A, Upadhye V, Harinath BC. A Prospective Study of Inhouse Developed SEVA TB ELISA Using Cocktail of Antigens and their Immunoglobulin in the Diagnosis of Tuberculosis Suspected Patients in a Tertiary Hospital Located in Rural Area. *Biomedical Research* 2009; **20(1);** 56-63.
17. Majumdar A, Upadhye V, Harinath BC. Peroxidase enzyme immunoassay for circulating SEVA TB ES-31 antigen in pulmonary tuberculosis sera. *Biomedical research* 2008; **19(3):** 201-06.
18. Nair ER, Banerjee S, Kumar S, Reddy MVR, Harinath BC. Isolation of *Mycobacterium tuberculosis* 31 kDa antigen protein of diagnostic interest from culture filtrate using anti ES-31 antibody by affinity chromatography. *Indian J Clin Biochem* 2001; **16**: 132-5.
19. Saha-Roy S, Shende N, Kumar S, Harinath BC. Effectivity of crude verss purified mycobacterial secretory protein as immunogen for optimum antibody production. *Ind J Exp Biol* 2005; **43**: 1196-8.
20. Upadhye V, Shende N, Kumar S, Harinath BC. Detec-tion of antibody and antigen in extrapulmonry tubercu-losis patients' sera using a cocktail of mycobacterial excretory secretory antigens and their antibodies. *Biomed Res* 2007; **18**: 161-6.
21. Sauar NJ, Foulkes JA, O'Neil PM. A comparison of alkaline phosphatase, beta galactosidase, penicillinase and Peroxidase used as labels for progesterone determination in milk by heterogenous microtitre plate. Enzyme immunoassay. *J Steroid Biochm* 1989; **33**: 423-6.

CHANCHAL SINGH MEMORIAL AWARD - 2010

The Tuberculosis Association of India awards every year a cash prize of Rs.1000/- to a medical graduate (non-medical scientists working as bacteriologists, biochemists, etc, in the field of tuberculosis included) who is below 45 years of age and is working in the field of tuberculosis, for an original article not exceeding 30 double spaced foolscap size pages (approximately 6,000 words, excluding charts and diagrams) on tuberculosis. Articles already published or based on work of more than one author will not be considered. Papers may be sent, in quadruplicate, to reach the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110001, before 30th June, 2010.

Original Article

CAN CORD FORMATION IN BACTEC MGIT 960 MEDIUM BE USED AS A PRESUMPTIVE METHOD FOR IDENTIFICATION OF *M. TUBERCULOSIS* COMPLEX?

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Summary

***Background*:** Serpentine cord formation in BACTEC MGIT 960 medium was evaluated as a rapid method for the presumptive identification of *M. tuberculosis* complex (MTBC).

***Material & Methods*:** Total 2527 samples were processed for AFB culture using MGIT 960 TB system over a period of three months. AFB smears were prepared from 1000 MGIT tubes flagged positive by the MGIT instrument and stained by ZN method to examine presence or absence of serpentine cording. The cord formation was compared with PNBA [p-nitro benzoic acid] test on MGIT system and all controversial cases were further evaluated by NAP [p-nitro-a-acetylamino-phydroxypropiophenone] test on BACTEC 460 TB system.

***Results & Discussion*:** Of the 1000 culture positives, 904 (90.4%) were identified as mycobacteria, of which 869 (96%) showed cording by smear microscopy. One (0.1%) was identified as nocardia. In the remaining 95 (9.5%) cases, primary smear made from MGIT vial was negative. Of 869 cultures showing serpentine cord formation, 842 were confirmed as MTBC and 27 as NTM by PNBA assay on MGIT 960 TB system. The sensitivity, specificity, positive and negative predictive values are found to be 99.6%, 54%, 96% and 91% respectively. An average detection time for PNBA assay was found to be eight days whereas cording results were available on the same day of culture positivity.

***Conclusion*:** Though highly sensitive it is not very specific and hence cannot be the only test for presumptive diagnosis of MTBC. **[*Indian J Tuberc 2010; 57:75-79*]**

Key words: Cord formation, Presumptive Identification, *Mycobacterium tuberculosis.*

INTRODUCTION

Isolation of mycobacteria by Acid Fast Bacilli (AFB) culture represents the corner stone on which definitive diagnosis of tuberculosis (TB) and other Non-Tuberculous Mycobacteria (NTM) disease relies. Most of the laboratories in the developing world rely on conventional Lowenstein and Jensen (L.J) media for culture followed by use of different biochemical tests for identification of mycobacteria, limitations of which are well known. Use of automated liquid culture systems like BACTEC MGIT 960, MB/Bact, Versa Tech is slowly increasing in disease endemic countries as India. These automated liquid culture systems, when combined with commercial molecular techniques like probe hybridization for species identification, are capable of producing positive results in two weeks or less for the vast majority of sputum smear-positive specimens, and within three weeks for smear-negative specimens.[1] However, such techniques are expensive, technically demanding and limited to a few clinically relevant species. Immuno chromatographic techniques such as CAPILIA are expensive and are still not available in India. Therefore in low-resource countries, many laboratories report a presumptive identification of *Mycobacterium tuberculosis* complex (MTBC) to physicians on the basis of a simple, rapid and cost-effective method i.e cord formation in liquid culture media.

Virulent strains of the MTBC, when grown in a liquid medium, often display characteristic serpentine cord formation.[2-4] Avirulent variants of MTBC grow in liquid media in a non-oriented,

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dispersed fashion.[3] NTM can form true cords in liquid culture but do so rarely, despite the fact that many species contain the cell wall glycolipid that mediates cord formation.[2,3] The interpretation of cording morphology, particularly in NTM such as *M. kansasii*, *M. avium complex, M. marinum, M. szulgai*, *M. chelonae*, *M. gordonae M. terrae*, and *M. phlei* that can frequently form looser aggregates or "pseudocords", is also subject to intero-observer differences.[2,3]

Cord formation has been advocated as a guide for the cost-effective utilization of DNA probes for the identification of *Mycobacterium* species[5], but to date only a few studies have evaluated the utility of cord formation for the presumptive identification of MTBC.[2,3,5-7] The present study was undertaken to determine the reliability of serpentine cording in BACTEC MGIT 960 medium as a rapid method to report the presumptive identification of MTBC.

MATERIAL AND METHODS

A total of 2527 consecutive clinical specimens were processed for AFB culture using MGIT 960 TB system over a period of three months (May 2009 to August 2009). All contaminated clinical specimens were digested and decontaminated by the standard *N*-acetyl-L-cysteine-NaOH method.[8] The sediment was suspended in 1 ml of sterile phosphate-buffered saline (pH 6.8). 0.5 ml of the processed specimen was then inoculated into MGIT 960 vials supplemented as described by the manufacturer, and 0.2 ml onto L.J medium slants. CSF and specimens collected from sterile sites were inoculated directly to MGIT vials. All inoculated MGIT vials were incubated in the MGIT 960 instrument either till they were flagged positive by the instrument or for a maximum of six weeks. L.J medium slants were examined daily for the first one week and thereafter, biweekly, for twelve weeks, for the visible appearance of colonies. Of the total 2527, 1000 MGIT vials were flagged positive by MGIT 960 TB system and checked for cording by ZNCF staining by two different observers. All controversial results were further rechecked by an experienced microbiologist. All positive cultures were further subjected to identification by p-nitro benzoic acid (PNBA) assay on MGIT 960 TB system.[9-13] All the MGIT vials flagged positive by machine but AFB negative by smear microscopy were further incubated at 37^0C and smear was repeated periodically after every three days. Obvious turbidity in the MGIT vial was confirmed by Gram staining of the smear as well as subculture on blood agar medium. In addition, 0.2 ml of positive broth was subcultured on an additional L.J. slant. Growth on this L.J subculture was used to rule out mixed infection, of MTB and NTM strains.

Serpentine cords were defined as ropelike aggregates of AFB in which the long axes of the bacteria paralleled the long axis of the cord.

Identification using PNBA

It has been reported that the growth of MTB isolates is inhibited by PNB 500 g/ml whereas NTM are resistant to this concentration. The PNB stock solution was prepared to ensure a final concentration of 500 mg/ml in the MGIT vial.[9-13] This stock solution was aliquoted and stored at -20^0. The PNBA test was performed by inoculating the positive culture into two MGIT tubes with and without PNBA and incubated in the MGIT 960 system. The growth Control (GC) was flagged positive by the MGIT system when Growth Unit reached 400. Cut off values of less than or equal to 100 was taken as sensitive indicating the growth of *M. tuberculosis* complex. Any value more than 100 was considered resistant indicating NTM. All cultures showing growth of NTM were further confirmed by r-nitro-a-acetylamino-b-hydroxypropiophenone (NAP) test in BACTEC 460 TB system.[14]

RESULTS

Of the total 2527 clinical specimens processed, 1000 (39.57%) were positive by MGIT 960 TB system (Table 1) which were further analysed for cording by AFB smear, and further processed for identification using PNBA test. These specimens included 742 respiratory specimens (650 Sputum, 62 Brochoalveolar lavage or BAL, 06 tracheal secretions, 24 pleural fluid) and 258 non-respiratory specimens (28 lymphnode, 25 tissue, 84

pus and aspirates, 07 body fluids, 20 CSF, 16 urine, 29 Abcess , 33 biposy , 16 Others). Of these 1000 specimens flagged positive by MGIT, 904 were positive for mycobacteria, one was showing Nocardia by smear microscopy. In the remaining 95 cases, primary smear made from MGIT vial was negative.

As shown in Tables 2 & 3, all 904 MGIT positive cultures were checked for cording by ZNCF method by two different observers. 845 were positive by observer 1 and 840 were positive by observer 2. Cords were recorded by observer 1 in 97.23% (n=845) whereas by observer 2 in 96.66% (n = 840). Final rechecking of controversial results by experienced microbiologist confirmed cord formation in 869/904 (96%) of MGIT positive cultures. Of 869 cultures showing serpentine cord formation, 842 were confirmed as MTBC and 27 as NTM by PNBA assay on MGIT 960 TB system. Of the 35 cultures negative for cording by ZNCF microscopy, three were found to be MTBC and 32 NTM by PNBA Test. Confirmation of all NTM by NAP test did not show any change in the results. Overall sensitivity, specificity, positive and negative predictive values are 99.6%, 54%, 96% and 91%

Table 1: Distribution of the total clinical specimens analysed

Total specimens analysed	2527
Specimens reported no growth (Negative) by MGIT	1527
Specimens flagged positive by MGIT	1000
Specimen Positive For Nocardia	1
AFB smear positives for mycobacteria	904
MGIT Positive Contamination	52
MGIT positives smear negative	43

Table 2: Comparison of PNBA and Cord Formation

N = 904	**No. of Samples showing Cording (869)**	**No. of Samples Absent for Cording (35)**	**Final Result**
PNBA Test Positive for MTBC (845)	842 (99.6%)	3 (0.4%)	MTB
PNBA Test Negative for MTBC (59)	27 (46%)	32 (54%)	NTM

Sensitivity = 99.6%
Specificity = 54%

Table 3: Comparison between two observers' readings for MTBC

N = 869	Cording Present	Cording Absent
Observer 1	845	24
Observer 2	840	29

respectively. An average detection time for PNBA assay was found to be eight days whereas cording results were available on the same day of culture positivity.

Of the 95, MGIT positive and AFB smear negative cases, 52 were identified as contamination. All re-inoculated MGIT vials after decontamination showed no growth. In the remaining 43 cases, organisms were not apparent by smear microscopy even after incubation till six weeks so all these were finally reported as no growth. All these were considered as MGIT false positives.

DISCUSSION

Rapid diagnosis of TB is critical to control of the disease; therefore, use of the most rapid methods available for culture and identification of MTBC is advocated. Cord formation has been reported as a simple, cost effective method for rapid presumptive identification of mycobacteria cultivated in liquid medium.[2,3,5,6] We evaluated the characteristics of cord formation of MTB complex in the liquid MGIT medium and results were compared with PNBA identification assay on MGIT. All NTM identified by PNBA method were further rechecked by NAP assay on BACTEC 460 TB system.

Of the 1000 MGIT tubes flagged positive by the MGIT 960 instrument, only 904 (90.4%) were identified as mycobacteria of which 869 (96%) showed cording by smear microscopy. One (0.1%) was identified as nocardia. Of the remaining 95 (9.5%) MGIT positive AFB smear negative cases, 52 were contaminated and 43 were AFB smear and culture negative for mycobacteria or other bacteria (at the end of the 42-day protocol). Reason for false positives can be depletion of oxygen due to other live cells present in the samples e.g. pus cells or cells present in tissues.

Gram positive cocci were the prevalent organisms responsible for contamination of MGIT 960 tubes in 52/2517 (2%) cases. Being highly enriched media contaminants like gram positive cocci can easily grow and utilized the oxygen in the medium giving false positive fluorescence signal. Hence smear preparation of all MGIT tubes flagged positive by MGIT will be helpful before processing it for further identification.

Studies conducted on the utility of cord formation for presumptive identification of MTBC, yield discordant data with sensitivity ranging from 22.9% to 90%.[2,3,5,6] Cording was found to be very specific for MTBC by Yagupsky *et al* and Morris *et al*.[3,6] However, in the present study, 27/59 (46%) NTM showed cording were misinterpreted as MTBC, decreasing the specificity of this test to 54%.

Cord formation is strictly an *in vitro* phenomenon and the proportion of isolates that demonstrate this phenomenon has been shown to vary greatly between clinical labs. The factor responsible for cord formation has been identified as trehalose 6,6'-dimyolate (TDM), a glycolipid with two long chain b-hydroxyl-a-branched fatty acids of variable length. TDM is a virulence factor and has been detected in NTM including MAC which rarely forms true cords, suggesting that the glycolipid is not sufficient for this property. Alternatively, the specific length of the fatty acid chains in TDM or species specific interaction with other cell wall components may determine its tendency to promote cord formation.

In the present study, the basis of microscopic morphology is available, on average, eight days earlier than the presumptive identification provided by the PNBA or and five days earlier than NAP differentiation test.

It should be noted that recognition of true cording sometimes would be difficult for the

microscopist. The loose, incomplete pseudocords produced by NTM may be misinterpreted as true cording. This is reflected by the differences in observer one and two's results (Table 3). In the present study, cord formation was not seen in three MTBC culture isolates by both the observers.

In a study carried out by McCarter *et al*, 54% MTBC from specimens incubated in liquid media for less than seven days did not show cording indicating a sufficient length of time is required to permit cord formation to develop.[2] Various other factors that can attribute to false negative results are strain differences, culture composition, growth conditions, differences in the handling of culture prior to ZNCF staining, number of fields observed and experience of the microscopist. The interpretation of cording morphology, particularly in NTM, is also subject to inter-observer differences.

CONCLUSION

The evaluation of cording provides rapid preliminary information before the results of other identification methods are available. Though highly sensitive, it is not very specific and hence cannot be the only test for presumptive diagnosis of MTBC. Before cord formation is used for presumptive identification, laboratory workers must be aware of the criterion. This method can be used in deciding how to progress with identification method but should not be used to generate preliminary reports to physicians.

REFERENCES

1. Sharp SE, Lemes M, Sierra SG, Poniecka A, Poppiti RJ Jr. Lowenstein-Jensen media. No longer necessary for mycobacterial isolation. *Am J Clin Pathol* 2000; **113**: 770-73.
2. McCarter, Y. S., I. N. Rarkiewicz, and A. Robinson. Cord formation in BACTEC medium is a reliable, rapid method for presumptive identification of *Mycobacterium tuberculosis* complex. *J Clin Microbiol* 1998; **36**: 2769–71.
3. Yagupsky, P. V., D. A. Kaminski, K. M. Palmer, and F. S. Nolte. Cord formation in BACTEC 7H12 medium for rapid, presumptive identification of *Mycobacterium tuberculosis* complex. *J Clin Microbiol* 1990; **28**:1451–53.
4. Middlebrook, G., R. J. Dubos, and C. Pierce. Virulence and morphological characteristics of mammalian tubercule bacilli. *J Exp Med* 1947; **86**:175–84
5. Kaminski, D. A., and D. J. Hardy. Selective utilization of DNA probes for identification of *Mycobacterium* species on the basis of cord formation in primary BACTEC 12B cultures. *J Clin Microbiol* 1995; **33**:1548–50.
6. Morris, A. J., and L. B. Reller. Reliability of cord formation in BACTEC media for presumptive identification of mycobacteria. *J Clin Microbiol* 1993; **31**:2533–34.
7. Badak FZ, Goksel S, Sertoz R, Guzelant A, Kizirgil A and Bilgic A. Cord formation in MB/Bactec TB medium is a Reliable Criterion for Presumptive Identification of *Mycobacterium* tuberculosis complex in Laboratories with High Prevalence of *M. tuberculosis*. *J Clin Microbiol* 1999; **37**:4189-91.
8. Kent and Kubica GP. Public health mycobacteriology: a guide for level III lab. Atlanta, GA: U.S. Department of health and human services, Public health services. Centre for disease control. 1985, 64-8.
9. C. Rodrigues, S Shenai, M Sadani, N Sukhadia, M Jani, K Ajbani, A Sodha, A Mehta. Evaluation of The BACTEC MGIT 960 TB system for recovery and identification of *M. tuberculosis* complex in a high through put tertiary care centre. *Indian Journal of Medical Microbiology* 2009; **27(3)**: 317-21.
10. Salman H. Siddiqi, Ph.D. (BD Fellow, Sparks, Maryland, USA) , Sabine Rüsch-Gerdes, Ph.D. (Director, National Reference Center for Mycobacteria, Borstel, Germany) 2006. BACTEC™ MGIT 960™ TB System Manual.
11. Barrie JD. Use of thiosemicarbazone and para-nitrobenzoic acid in screening tests for anonymous mycobacteria. *J Clin Path* 1967; **20**: 86-8.
12. Giampaglia CMS, Martins MC, Inumaru VTG, Butuem IV and Telles MAS. Evaluation of a rapid differentiation test for the *M. tuberculosis* complex by selective inhibition with p-nitrobenzioc acid and thiophene-2-carboxylic acid hydrazide. *Int J Tuberc & Lung Dis* 2005; **9(2)**: 206-09.
13. Giampaglia CMS, Martins MC, Chimara E. *et al.* Differentiation of *Mycobacterium tuberculosis* from other mycobacteria with p-nitrobenzoic acid using MGIT 960. *Int J of TuberC & Lung Dis* 2007; **11(7)**: 803-07.
14. Rodrigues C, Shenai S, Almeida D, Sadani M, Vadher C and Mehta A. Use of BACTEC 460 TB system in the diagnosis of tuberculosis. *Indian Journal of Medical Microbiology* 2007; **25(1)**: 32-5.

Original Article

RANDOMIZED, DOUBLE-BLIND STUDY ON ROLE OF LOW LEVEL NITROGEN LASER THERAPY IN TREATMENT FAILURE TUBERCULAR LYMPHADENOPATHY,SINUSES AND COLD ABSCESS

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Summary

Background: Effectiveness of low level nitrogen laser therapy along with antitubercular treatment (ATT) in cases of treatment failure and drug resistant tubercular lymphadenopathy, sinuses and cold abscess.

Methods: In a double-blind randomized controlled trial of LLLT ,104 patients assigned to either the low level nitrogen laser therapy along with ATT (LLLT group) (n =54) or ATT only(Chemotherapy group)(n=50). Both groups were treated two times per week for five weeks. Those in the treatment group received pulse nitrogen laser with a pulse duration of seven nanosecond, wave length 337 nanometer and average power output of 5 mW whereas those in the control group were treated with sham laser. The primary outcome measure was bacteriological conversion and the secondary outcome measures were decrease in size of lesion and the clinical improvement.

Results: Acid Fast Bacilli (AFB) smear, AFB culture and Polymerase Chain Reaction(PCR) conversion rate at five weeks (after 10 sittings of laser) were 49.15%(Fishers P exact test-p= 0.015), 60%, 44.44% (Fishers P exact test-p= 0.048) in LLLT group as compared to 11.86%,20%,17.77% in chemotherapy group. Average percentage reduction in the size of gland at 5 weeks was 70.67% (p value 0.01) as compared to 54.81 in chemotherapy group. Average time taken for closure of sinuses was 11.03 weeks in LLLT group as compared to 26 weeks in chemotherapy group. The follow up was conducted for two years.

Conclusion: Low level nitrogen laser therapy can be used as an adjunctive therapy along with antitubercular drugs in cases not responding and drug resistant tubercular lymphadenopathy, sinuses and cold abscess. **[*Indian J Tuberc 2010; 57:80-86*]**

Key words: Laser, Lymph node tuberculosis, Drug resistant

INTRODUCTION

Tuberculosis affects more than eight million people every year and has serious repercussions on economy, as well as the psychologic and social status of the affected individuals. It has therefore been declared a global emergency in 1993 by World Health Organization (WHO). Since then, significant developments have taken place in the treatment and control of tuberculosis. One notable advancement has been the implementation of the Directly Observed Treatment, Short course (DOTS) along with fixed dose combination of existing drugs. However, the currently available therapeutic regimens have inherent disadvantage of long treatment duration, which often leads to patient non-compliance and the risk of drug resistance. Hence, new modalities of potent treatment which reduce the treatment period and also active against resistant strain are needed to combat this disease.

The present study has been carried out to study efficacy and safety of 10 sittings of Low Level Nitrogen Laser Therapy (LLLT) in the management of treatment failure tubercular lymphadenopathy, sinuses and cold abscess.

MATERIAL AND METHODS

Double-blind randomized controlled trial study on role of low level nitrogen laser therapy in treatment failure tubercular lymphadenopathy,

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sinuses and cold abscess has been studied for a period of three years from January 2005 to December 2007 and follow up was done for a period of two years. After getting approval from the Ethical Committee of the institution, the study was started.

All patients gave a detailed medical history and received a physical examination. All patients gave an informed consent to participate in the study.

The criteria for inclusion of patients in the present study were: 1) Antitubercular treatment (ATT) for more than six months showing no response 2) Acid fast bacilli (AFB) grown in culture after six months of ATT 3) Abscess formation showing no response to treatment 4) Sinus tract formation showing no response to treatment 5) Age group more than 15 years but less than 65 years with a diagnosis of drug resistant tubercular lymphadenopathy and 6) DOTS (Directly Observed Treatment, Short Course) Category-II failure.

Exclusion criteria included patients of 1) Human immunodeficiency virus(HIV) positive 2) Hepatitis B surface antigen positive 3) Diabetes Mellitus and 4) Renal disease.

Routine tests included complete haemogram, blood sugar, screening test for HIV, hepatitis B surface antigen, Mantoux test and sputum examination for AFB.

In patients of lymph node abscess, sinus and cold abscess, we have used microbiological analysis for the presence of *Mycobacteria* in the lymph node tissue. AFB smear microscopy was done by flourescent technique, AFB culture was done on Lowenstein Jensen slopes (L-J), Polymerase Chain Reaction (PCR) by professional biotech kit using RNA probe. In all patients, Fine Needle Aspiration Cytology (FNAC) was done.

In both groups, size of lymph node was measured, vertical plus horizontal, by measuring scale at baseline and after 1st and 5th weeks, during laser therapy and follow up measurement was done at 10th and 24th weeks of treatment by investigator.

Similarly, in both groups, aspirate from the lymph node was subjected to AFB smear, AFB culture sensitivity, PCR at baseline, after 1st and 5th weeks of laser therapy. Microbiologist was unaware of the nature of treatment patient was receiving. Follow up AFB smear and AFB culture sensitivity was done at 10th week. In 19 patients, microbiological analysis was not done because of solid nature of lesion.

In both groups, antitubercular treatment was given according to AFB culture and sensitivity report where culture was positive, empiric therapy was given where AFB was not grown in culture. In both groups, ATT was given for two years beyond AFB conversion.

Eligible patients were randomly assigned to the LLLT group and chemotherapy group by a study coordinator who also turned off the machine in the sham treatment arm. To ensure a double-blind study noise of laser machine was inaudible in presence of noise of vacuum pump. In LLLT group, both the laser machine and vacuum pump were started while in case of chemotherapy group only vacuum pump was started (Fig. 1).

The Jelco canula (16 gauge , 50 mm length) was first introduced inside the lesion, pus was aspirated and through the canula , the fiber of the laser equipment was introduced inside the lesion. At each session, in cases of LLLT group, laser was delivered for 780 seconds, while in cases of chemotherapy group sham irradiation was done This was performed twice per week, for a total of ten sessions. No anesthesia was given.

Laser used in this study was a pulse laser with a pulse duration of seven nanosecond wave length 337 nanometer and average power output of 5 mW at the tip of the fiber. The laser device was manufactured by Raja Ramanna Centre for Advanced Technology,Indore,India.

Primary outcome measure was frequency of AFB smear conversion, culture conversion, PCR for *M.TB* complex conversion in aspirated pus at 1st and 5th weeks of laser therapy. Secondary Average

outcome measure was 1) Average time taken for resolution of fever and 2) Average percentage reduction in size of lesion.

Statistical analyses were based on the intention-to-treat principle.

RESULTS

Epidemiology

A final total of 104 cases were included in the study: Males 33 (31.73%) and females 71 (68.26%). Male to female ratio was 1:2.1. Mean age of the patients was 25.2 years (Range 15-65) in LLLT group as compared to 26.9 years in chemotherapy group. Average duration of illness, before starting treatment, was 17.65 months as compared to 11.82 months in chemotherapy group. Both groups were randomly distributed according to type of lesion (Table 1).

Diagnostic findings

In 75 (72.11%) patients, the tissue samples showed chronic granulomatous inflammation with caseating necrosis. Of the 85 patients, 59 (69.41%) had AFB smear positive, in 11 (12.94%) patients, AFB was grown in culture and in 45 patients, PCR for *M.TB* complex detected. Out of 104 patients, in 90 (86.53%) patients, Mantoux Test was more than 10mm in size (Table 2).

Laser therapy

Average time taken for disappearance of fever in LLLT group was 6.25 weeks as compared to 8.07 weeks in chemotherapy group.

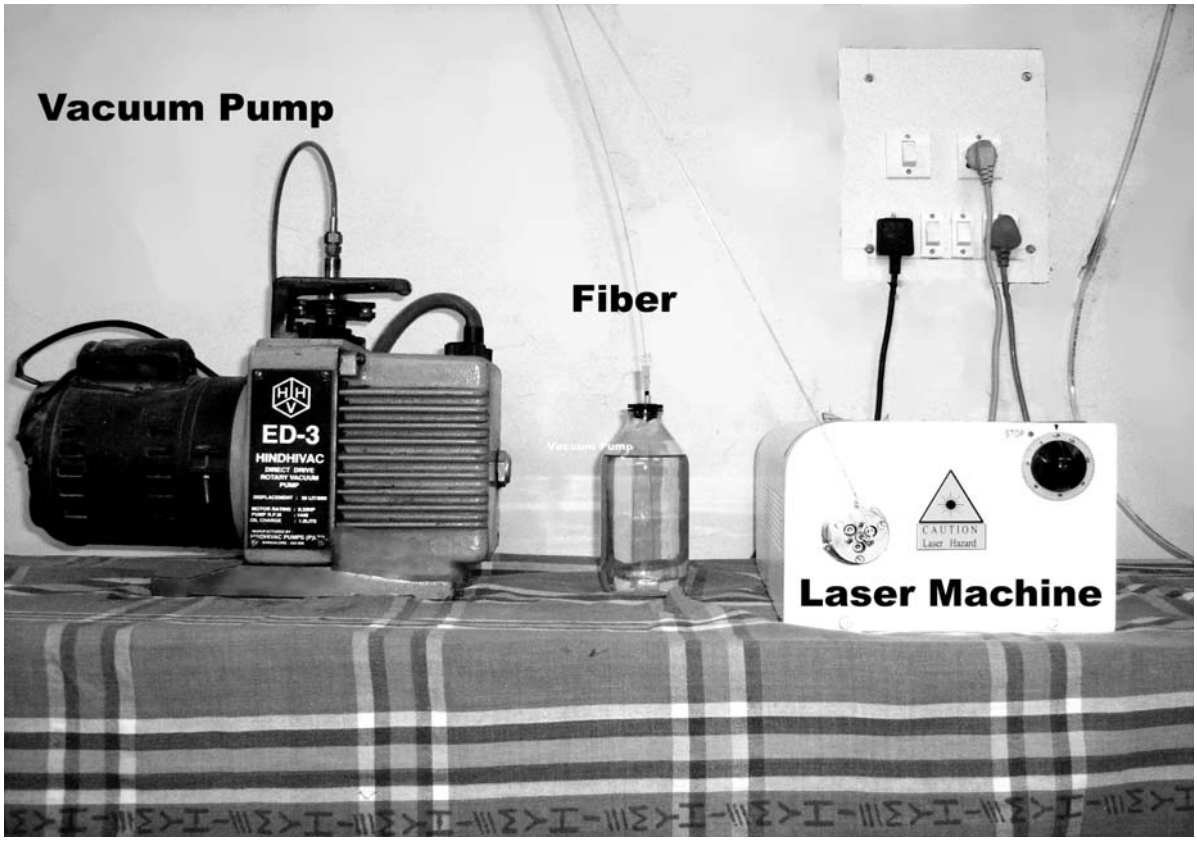

Fig. 1: Laser Machine with Fiber and Vacuum pump

Table 1: Distribution of patients according to lesion among LLLT and Chemotherapy group (n =104)

Type of lesion	No. of patients (LLLT group)(n=54)	No. of patients (Chemotherapy group)(n=50)
Lymphnode	9 (17.33)	10(20.00)
Lymphnode abscess	6(11.55)	6(12.48)
Lymphnode abscess with discharging sinus	25 (48.14)	23(47.84)
Cold abscess	14 (26.96)	11 (22.88)

Table 2: Diagnostic criteria of the104 patients included in the study

Diagnostic criteria	Total no of patients In which test was done	No. of pts. showing positive results	Percentage (%)
AFB Smear	85	59	69.41
AFB Culture	85	11	12.94
PCR for M.TB Complex	85	45	52.94
FNAC	104	75	72.11
Mantoux Test	104	90	86.53

Table 3: Average percentage decrease in the size of lymph node and abscess according to duration in weeks among LLLT and Chemotherapy group (n=104)

Group	1st wk	5th wks	10th wks	24th wks
LLLT	33.77	70.67	86.65	97.12
Chemotherapy	22.98	54.81	69.62	86.51

At 5 weeks t: 2.646, p value 0.01

Table 4: Number of patients showing AFB smear conversion according to duration in weeks among LLLT and Chemotherapy group (n=59)

Group	1st wk	5th wks	10th wks
LLLT	16 (27.11%)	13(22.03%)	1(1.69%)
Chemotherapy	2(3.38%)	5(8.47%)	7(11.8%)

Fishers exact test at 5th week p= 0.015

Table 5: Number of patients showing AFB culture conversion according to duration in weeks among LLLT and Chemotherapy group (n=11)

Group	1st wk	5th wks	10th wks
LLLT	3(30%)	3(30%)	1 (9.09%)
Chemotherapy	1(10%)	1(10%)	2(20%)

Table 6: Number of patients showing PCR conversion according to duration in weeks among LLLT and Chemotherapy group (n=45)

Group	5th wks
LLLT	20(44.44%)
Chemotherapy	8(17.77%)

Fishers exact test-p= 0.048

weight gain in patients of LLLT group was 4.51 kg as compared to 3.14 kg in chemotherapy group.

Average percentage reduction in the size of lymphnode, abscess (Fig. 1) and cold abscess at five weeks (after 10 sittings of laser) was 70.67 in cases of LLLT group as compared to 54.81 in chemotherapy group (Table 3). In LLLT group, lymph node and abscess disappeared completely in 23.4 weeks and sinus was closed in 11.03 weeks as compared to 48.1 and 26 weeks in chemotherapy group.

AFB smear, AFB culture and PCR conversion rate at five weeks were 29 (49.15%), six (60%) and 20 (44.44%) in LLLT group as compared to seven (11.86%), two (20%) and eight (17.77%) in chemotherapy group (Tables 4,5 and 6).

In LLLT group (after 10 sessions of laser therapy), greater reduction in the size of lesion (p value 0.01) and early bacteriological conservation (p value 0.01) was seen as compared to chemotherapy group.

DISCUSSION

The standard therapy of treatment failure tubercular lymphadenopathy, abscess, sinus, and cold abscess is antitubercular drugs along with surgery. According to Dharma Kanta *et al*, treatment of tubercular cervical lymphadenopathy leading to ulceration / sinus formation, excision of ulcer / sinus along with excision of underlying caseating lymphnodes were followed by short course of antitubercular chemotherapy[1]. Similarly, results were observed by Siu *et al* where they suggested that all easily assessable tuberculous lymph nodes should be removed and that persistent discharging sinuses should be treated by surgery[2].

Peripheral lymph node tuberculosis is the most common form of extra pulmonary tuberculosis. Cervical tubercular lymphadenopathy, lymph node abscess with discharging sinus is still the most common cause of persistent cervical lymph node enlargement in the developing countries.

In the present study, we have taken those cases who already had taken ATT for more than six months along with surgical excision, showing no response to treatment. In our knowledge, we have not found any study in which simultaneous monitoring of decrease in the size of lesion along with microbiological study has been carried out.

Low proportion of positive cultures in our study is due to the presence of bacteriostatic substances in tubercular lymph node, which inhibit the growth of bacilli *in vitro*, bacilli in the glands being scanty. The low rate could also be due to patients having already taken ATT for more than six months. Among culture positives, most common drug resistance was found to be of Isoniazid followed by Pyrazinamide and Rifampicin.

Three patients of lymphadenopathy did not respond because lymphnodes were multiple and deep seated. One patient of sinuses had recurrence because of multiple sinuses. All four patients required surgical excision. In rest of the patients, no recurrence of the lymphnode and no discharge from the sinus were seen during two years of follow up.

The exact mechanism how LLLT works is not known, possibly LLLT enhances immune system; inhibits growth of the tubercular bacilli,

increases circulation to the site of lesion to deliver more drugs.

Influence of laser on the immune system has been evidenced in medical literature. Immunological effects on leukocytes, T & B cells and NK lymphocytes, macrophages result in local and systemic effects through a complex mechanism of action, which is not yet definitively elucidated. *In vitro* experiments have also provided some evidence as possible influence of nitrogen laser irradiation on the immune system, for example nitrogen laser irradiation, was seen to enhance the intracellular killing of internalized bacteria in human neutrophil[3].

The high intensity focused nitrogen laser irradiation has been shown to lead to direct inhibition of bacteria[4]. *In vitro* experiment of UVA radiation from Nitrogen laser irradiation on tubercle bacilli at 337 nm,average power 2.0 mW was observed to cause a dose-dependent decrease in cell viability due to significant change in fluidity of lipid regions in the cell wall of laser exposed cells[5].

Twice per week was based on *in vitro* report of effect of nitrogen laser irradiation (337 nm) on viability of clinical isolates of *Mycobacterium tuberculosis*. Bacteria were exposed to a nitrogen laser (average power 2.0 mW) *in vitro* at power density of 70 +/- 0.7 W/m2 for 0-30 min, and the cell viability was determined by luciferase reporter phage (LRP) assay. Immediately after laser exposure, all the clinical isolates investigated showed a dose-dependent decrease in cell viability. However, when the laser-exposed isolates were incubated in broth medium for three days, most of these showed significant recovery from laser-induced damage[5]. Previous study on effect of LLLT(337 nm, average power 2.0 mW twice per week)on treatment failure tubercular lymphadenopathy[6] and drug resistant pulmonary tuberculosis[7] showed significant results in laser treated group.

In continuation of the pioneering work of Finsen on treatment of skin TB by Ultra Violet (UV) light[8] and *in vitro* reports on bactericidal effect of UV light on tubercular bacilli[9], Eshanchanov *et al* reported the use of UVA radiation from nitrogen laser (337-nm) for the treatment of patients with Pulmonary Tuberculosis[10]. Ethne L *et al* and J. Stephen Guffey *et al* also observed bactericidal effects of LLLT on bacteria[11,12]. *In vitro* exposure of UVA radiation has been reported to lead to alteration in cell membrane properties via damage to membrane lipids in Escherichia coli[13-15].

Only a few studies and isolated case reports describing the role of low level laser therapy in pulmonary Tuberculosis[16-18] tubercular lymphadenopathy[19,20,6] are available in the literature.

CONCLUSION

In our study, LLLT has given encouraging results both in the faster healing of the lesion and clearance of tubercle bacilli among LLLT group as compared to chemotherapy group. More studies are needed to determine exact dose and duration of treatment.

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REFERENCES

1. Baskota DK., Prasad R., Sinha BK., & Amatya RC. Frequency in effective treatment of ulcers and sinuses in cases of tuberculous cervical lymphadenitis. *JCPSP* 2005; **15(3)**: 157-9.
2. Siu KF, Ng A, Wong J. Tuberculous lymphadenopathy : a review of results of surgical treatment. *Aust NZJ Surg* 1983; **53** :253-7.
3. Sachdeva, R., Bhagwanani, N.S., Chitnis, D.S. Low incident energy levels enhance the biocidal activity of

human neutrophils on internalized bacteria : an *in vitro* study. *Laser therapy* 1995; **7**: 107-12.

4. Sachdeva, R., Bhagwanani, N.S., Chitnis, D.S. The nitrogen laser inhibits the growth of wide range of microbes *in vitro*. *Laser therapy.* 1995; **7**: 23-6.
5. Dube A, Jayashankar K, Prabakaran L, Kumar V, Gupta P.K. Nitrogen laser irradiation (337 nm) causes temporary inactivation of clinical isolates of *Mycobacterium tuberculosis*. *Laser in Medical Science* 2004; **19**: 52-6.
6. Bajpai A, Bhargava S, Gupta P.K. and Jain NK. Role of low level laser therapy in treatment failure Tubercular lymphadenopathy. *Indian J Tuberc* 2006; **53**: 229-31
7. Bajpai A, Bhargava S ,Jain NK and Gupta PK. Role of low level laser therapy in drug resistant pulmonary tuberculosis *Indian J Tuberc* 2006; **53**:135-40.
8. Finsen N.R. The chemical rays of light and smallpox. In: Finsen NR (ed) Phototherapy. 1901; Anold, New York (J.H.Se-qiuera, translator)
9. Dreher W, Domanska B. Bactericidal effect of ultraviolet rays of the quartz lamp on tuberculi bacilli H37Rv and on acid fast bacilli cultured from sputum of a patient. *Gruzlica* 1968; **36**: 181-3.
10. Eshankhanov M, Khodzhaeva MI, Takhirov ON, Priluk K P. Nitrogen laser in the therapy of lung destructive tuberculosis. In : Proceedings of the international conference, Tashkent, 1989; part 3, p 206.
11. Ethne L, Nussbaum, Lothar, Lilge, Tony Mazzulli. Effects of Low Level Laser Therapy (LLLT) of 810 nm upon *in vitro* growth of bacteria : Relevance of irradiance and radiant exposure. *Journal of clinical laser medicine and surgery* October 1, 2003 , **21(5)**: 283-90.
12. J.Stephen Guffey, Jay Wilborn: *In vitro* bactericidal effects of 405-nm and 470-nm blue light. *Photomedicine and laser surgery* 2006; **24(6)**: 684-8.
13. Mody R, Mody B, Dave P. Damage to the plasma membrane in Escherichia coli K-12 induced by far – ultraviolet radiation and its repair. *Radiat Res* 1991; **127**: 156-63.
14. Pizarro RA. Once LV. Membrane damage and recovery associated with growth delay induced by near UV radiation in Escherichia coli K-12 Photochem. *Photobiol* 1988; **47**: 391-7.
15. Chamberlain J. Moss SH. Lipid peroxidation and other membrane damage produced in Escherichia coli K 1060 by near UV radiation and deuterium oxide. *Photochem Photobiol* 1987; **45**: 625-30.
16. Puri MM, Arora VK. Role of gallium arsenide laser irradiation at 890 nm as an adjunctive to anti-tuberculosis drugs in the treatment of pulmonary tuberculosis. *Indian J Chest Dis Allied Sci* 2003; **45(1)**: 19-23.
17. Abashev IM, Kozlova AI. Role of external laser radiation in the multi-modality treatment of patients with destructive pulmonary tuberculosis. *Probl Tuberk* 1996; **(6)**: 54-7.
18. Kiryanova V., Vinogradova T., Levashov A., Potepunp effectiveness of blue light laser in complex treatment of Pulmonary Tuberculosis. *Photodiagnosis and Photodynamic Therapy August 2008;* **5:** S21-S22.
19. Puri M.M , Singla R, Jaiswal A, Gupta K, Jain RC . Case reports on the role of laser therapy in the treatment of tuberculosis of the lymphnodes. *Laser therapy* 1997; **9**: 55-8.
20. Makarova UE. Laser therapy in complex treatment for early tuberculosis of peripheral lymph nodes. *Probl Tuberk Bolezn Legk* 2008; **6:**15-8.

Indian J Tuberc 2010; 57: 87-89

STATUS REPORT ON RNTCP*

RNTCP has achieved NSP case detection rate of 66% and treatment success rate of 87% at the national level during the fourth quarter, 2009. The low case detection in the fourth quarter is persisting; however the overall NSP case detection rate for the year 2009 is 72%.

RNTCP performance during fourth quarter, 2009

During the quarter, over 1.84 million suspects were examined, 210,886 sputum positive cases were diagnosed, and 356,620 TB cases were registered for treatment. The annualized total case detection rate is 123 cases per 100,000 population. With a total of 143,608 new smear positive cases being registered for treatment, the new smear positive TB case detection rate (annualized) for the fourth quarter 2009 is 66%. In addition to this, 91,576 new smear negative cases, 52,120 new extra pulmonary cases, 46,953 smear positive re-treatment cases and 21,965 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 fourth quarter 2008 is 87% and the sputum conversion rate of patients registered during third quarter, 2009 is 90%. The default rates among NSP (5.5%), NSN (7%) and re-treatment cases (14%) continue to show the declining trend over the past several quarters.

Major Activities during the quarter

National Air born infection control guidelines

Air-borne infection control measures are imperative for preventing spread of TB and other airborne infections from person-to-person and also reducing the risk of spread to health workers in institutional settings. RNTCP has taken concrete steps towards this endeavour by developing the National

Population in India covered under DOTS and total tuberculosis patients put on treatment each quarter

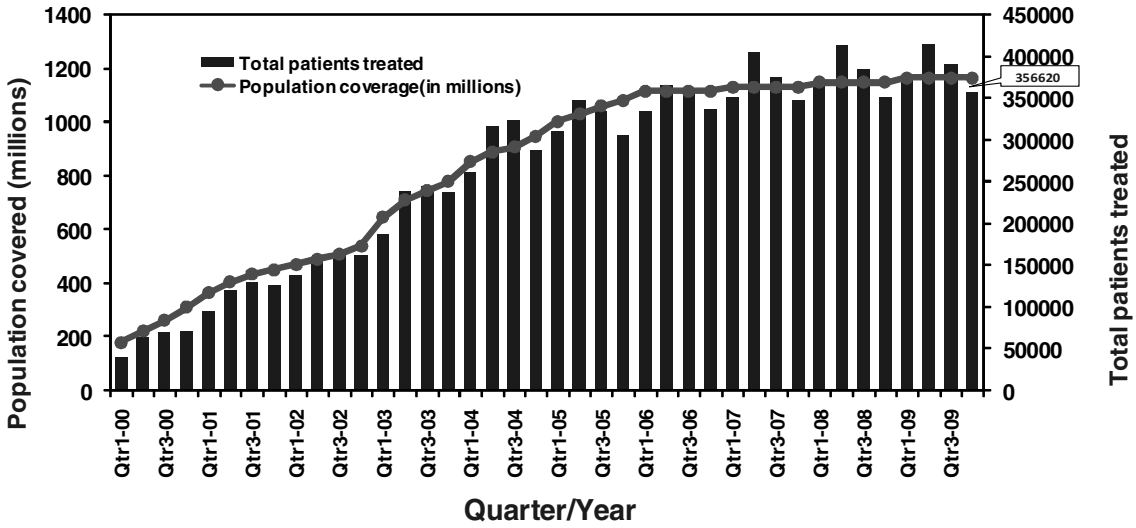

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Table: Performance of RNTCP Case Detection (2009, fourth quarter), Smear Conversion (2009, third quarter), and Treatment Outcomes (2008, fourth quarter)

State	Population (in lakh) covered by RNTCP[1]	Suspects examined per lakh population	No of Smear positive patients diagnosed[2]	Total patients registered for treatment[3]	Annualized total case detection rate	New smear positive patients registered for treatment	Annualized new smear positive case detection rate (%)		No of new smear negative cases registered for treatment	No of new EP cases registered for treament	No. of smear positive re-treatment cases registered for treatment	3 month conversion rate of new smear positive patients	Cure rate of new smear positive patients	Success rate of new smear positive patients
Andaman & Nicobar	4	235	99	204	194	71	68	90%	50	47	27	88%	85%	89%
Andhra Pradesh	830	167	18226	27540	133	11952	58	77%	7183	2961	4034	92%	87%	89%
Arunachal Pradesh	12	213	256	541	178	168	55	74%	159	85	63	92%	88%	89%
Assam	304	116	5246	9186	121	3853	51	68%	2576	1160	912	89%	85%	87%
Bihar	953	99	11374	20655	87	8735	37	49%	6753	1313	1802	88%	81%	90%
Chandigarh	11	319	378	548	201	185	68	72%	102	149	77	89%	92%	92%
Chhatisgarh	240	110	3022	6638	110	2493	41	52%	2563	889	425	89%	82%	87%
D & N Haveli	3	153	63	100	148	35	52	65%	29	17	12	89%	87%	87%
Daman & Diu	2	259	32	64	133	21	44	54%	14	10	7	100%	57%	71%
Delhi	176	236	5059	10345	235	2793	63	67%	1934	3018	1551	89%	87%	87%
Goa	17	200	308	481	115	180	43	54%	77	137	57	93%	86%	86%
Gujarat	572	181	13600	18891	132	8425	59	74%	2454	2553	3898	92%	88%	88%
Haryana	241	171	5152	8543	142	2927	49	51%	1812	1331	1835	90%	85%	86%
Himachal Pradesh	66	224	1632	2717	164	950	57	61%	516	586	497	93%	89%	90%
Jammu & Kashmir	128	151	1865	2871	90	1417	44	47%	456	542	365	93%	90%	91%
Jharkhand	304	109	4792	9044	119	3966	52	70%	3041	631	697	91%	86%	90%
Karnataka	580	196	10851	16603	114	6500	45	60%	3581	3257	2264	87%	80%	82%
Kerala	346	245	3684	6930	80	2801	32	65%	1632	1638	650	83%	81%	82%
Lakshadweep	0.7	89	0	3	17	0	0	0%	1	2	0	25%	100%	100%
Madhya Pradesh	705	113	10880	19797	112	7146	41	51%	6563	2284	2502	89%	85%	88%
Maharashtra	1083	155	18539	33946	125	12994	48	60%	7965	6127	3853	89%	84%	85%
Manipur	27	128	341	840	126	245	37	49%	286	153	76	87%	81%	82%
Meghalaya	26	190	609	1105	172	413	64	86%	208	252	144	86%	81%	83%
Mizoram	10	182	201	591	238	140	56	75%	165	167	45	89%	87%	92%
Nagaland	22	152	387	820	148	292	53	70%	205	153	93	91%	90%	91%
Orissa	403	135	6875	12006	119	5244	52	61%	2955	2135	999	88%	82%	86%
Puducherry	11	421	558	371	136	185	68	90%	63	74	42	90%	88%	88%
Punjab	269	157	5155	8385	125	3487	52	55%	1523	1596	1357	90%	86%	88%
Rajasthan	657	135	14289	24359	148	8498	52	65%	7069	3188	4469	92%	88%	89%
Sikkim	6	256	151	344	229	99	66	88%	74	91	51	84%	88%	88%
Tamil Nadu	669	262	11115	19986	119	7925	47	63%	5280	3992	2225	91%	86%	86%
Tripura	36	136	423	662	75	366	41	55%	114	99	64	92%	87%	88%
Uttar Pradesh	1944	148	38470	64051	132	26821	55	58%	18648	7183	8327	91%	86%	89%
Uttarakhand	96	165	2077	3076	128	1135	47	50%	748	442	582	89%	82%	85%
West Bengal	889	167	15177	24377	110	11146	50	67%	4777	3858	2951	88%	85%	86%
Grand Total	**11641**	**158**	**210886**	**356620**	**123**	**143608**	**49**	**66%**	**91576**	**52120**	**46953**	**90%**	**85%**	**87%**

1 Projected population based on census population of 2001 is used for calculation of case-detection rate. 1 lakh = 100,000 population

2 Smear positive patients diagnosed, include new smear positive cases and smear positive retreatment cases

3 Total patients registered for treatment, include new sputum smear positive cases, new smear negative cases, new extra-pulmonary cases, new others ,relapse,failure,TAD and retreatment others

Guidelines on Airborne Infection Control in Health Care and Other Settings. A national workshop was organized at New Delhi in November 2009 to develop the National guidelines for Airborne infection control. The guidelines were developed by the National Airborne Infection Control Committee (NAICC), a multi-lateral group of national experts from renowned Medical Colleges of India, National Communicable Disease Control (NCDC) Institute, National AIDS Control Organization (NACO), Central TB Division (CTD), World Health Organization, Architects and Engineers.

Practical Approach to Lung Health (PAL)

PAL strategy has been identified in the Stop TB Strategy as a model to strengthen the health systems. This initiative is aimed at managing respiratory patients in primary health care settings while expanding TB detection and good-quality TB services. PAL focuses on the most prevalent respiratory diseases at first-level health facilities. A pilot project of PAL is being implemented in the state of Kerala. In order to develop the technical and operational guidelines for PAL, a workshop was organized in Trivandrum, Kerala on 21st and 22nd October 2009 with international, national and state experts with WHO support. The project implementation plan was developed by the state technical working group for PAL and the project will be implemented by State Disease Control and Monitoring Cell, NRHM Kerala with technical support of RNTCP.

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

DOTS Plus services for management of MDR TB have been rolled out in the states of Gujarat, Maharashtra, Andhra Pradesh, Haryana, Delhi, Kerala, West Bengal, Tamil Nadu, Rajasthan and Orissa. In this quarter, 443 MDR TB patients have been initiated on DOTS Plus treatment bringing the total number of MDR TB patients on treatment to 1412 in these states.

Progress in the involvement of NGOs and PPs

The second National Review workshop of IMA GFATM RNTCP PPM Project was conducted on 14.10.2009 at Hotel Atrium, Surajkund, Haryana. The NWG members, State Coordinators, IMA Technical Consultants, State Presidents and State Secretaries of the Project States and private doctors trained by IMA and involved in DOT, participated in the review. The National President of IMA Dr Ashok Adhao inaugurated the workshop. There was representation from CTD and State TB officers of the six Project states .

A RNTCP review of National Thermal Power Corporation Project sites was done on 5.12.2009 at New Delhi. DMC /DOT Centres are running in 10 hospitals of NTPC across the country. They have put 991 patients on treatment till December 2009. They provide ambulance for Advocacy, Communication and Social mobilization in the slum areas and for DOT.

Progress in TB HIV Collaborative Activities

Intensified TB/HIV package of Services has been rolled out in seven additional states – Punjab, Chandigarh, Rajasthan, Assam, Orissa, West Bengal and Kerala. The training of master trainers at state level has been completed in all these states except Rajasthan. The Intensified TB/ HIV package modules for all levels of staff have been revised and may be accessed from www.tbcindia.org. A new TB/HIV module for ART centre staff has been drafted and the training of national level master trainers in this module has been completed.

Progress in HRD related activities

Revision of Modules 1-8 which have been specifically re-designed for Programme Managers is underway. Detailed discussions for the same are taking place in Central TB Division for finalization of the same. This revision exercise is crucial to the programme as many significant changes are being included in this version, in addition to the fact that these modules are designed to address the needs of programme managers, thereby strengthening capacity building and managerial focus in the programme. Further, work on designing separate modules for Medical Officers and facilitators' guide is also being initiated by CTD.

Progress in ACSM

Communication material for community level activities for awareness generation and for use by the DOT providers for patient counselling has been finalized by the media agency, and will be available on RNTCP website by nest quarter.

Case Reports

PELVIC TUBERCULOSIS CONTINUES TO BE A DISEASE OF DILEMMA - CASE SERIES

S. Chhabra[1], K. Saharan[2] and D. Pohane[3]

(Received on 25..2.2009; Accepted after revision on 29.10.2009)

Summary: Tuberculosis (TB) has become a global epidemic again with emergence of HIV/AIDS and multi-drug resistant strains of TB. Female genital tuberculosis (GT) is typically a disease of young women and its occurrence in post menopausal women is rare. Amongst the genital disorders, GT is the most baffling especially because of its various presentations. So GT is notorious for evading diagnosis. A series of cases of females GT between the age 25 yrs to 40 yrs is being reported with women having spectrum of clinical features, creating diagnostic dilemma and so final diagnosis by histopathology after laparotomy. So a high degree of suspicion aided by intensive investigations may be required for the diagnosis of GT. Medical therapy is the main treatment, however some do need surgery. Research needs to be continued for early establishment of timely diagnosis of GT and modalities of effective therapies.***[Indian J Tuberc 2010; 57:90-94]***

Key words: Pelvic Tuberculosis, Genital.

INTRODUCTION

Globally 9.2 million new cases were reported and 1.7 million deaths[1] occurred due to tuberculosis (TB) in 2006. Reported incidence of TB in India, as per WHO, is 168/ 1,00,000 and there are about 28 deaths/lac population. TB has become global epidemic with emergence of HIV/AIDS and multidrug resistant strains of the microbes [2] . Amongst the female genital disorders, genital tuberculosis (GT) is the most baffling, especially because of its varied presentations. It tends to manifest as menstrual irregularities, infertility, chronic pelvic or lower abdominal pain or lump and is mostly acquired by haematogenous route. Genitourinary tuberculosis is mostly a secondary manifestation of primary pulmonary or abdominal tuberculosis[3-6]. Approximately, 30% of cases of extra-pulmonary tuberculosis involve the urogenital tract[7]. Although the spread of tuberculosis from primary infection to the pelvis usually occurs early, detection of genital infection is seldom feasible, because of varied presentation and is likely to have focal pathology in any organ. For these reasons, it is notorious for evading diagnosis. Female GT is typically a disease of young women and its occurrence in postmenopausal women is believed to be rare[8]. The prevalence of female GT varies from 1% to 19% depending on the country[6]. It is estimated that 5-13 % of the females presenting to infertility clinics in India have genital TB. GT frequently presents without symptoms & diagnosis requires a high index of suspicion, at least 11% of the patients lack symptoms and the disease is often detected during diagnostic work up of women attending infertility clinics. Early diagnosis before extensive genital damage occurs with appropriate treatment associated with a more favourable outcome with better chances for fertility also. The fallopian tubes are the first and most commonly affected part followed by endometrium, ovary and cervix. Adhesions between tubes, ovaries, omentum, intestines, liver, and diaphragm are common findings (Fitz Hugh Curtis syndrome).The clinical picture is so variable that single symptoms or signs or investigation may not be suggestive. There may not be any clinical evidence that the fallopian tubes (most common site of involvement) are involved but minimal or subclinical disease is present. Female GT can also present as an abdominal mass with raised levels of CA 125 and can masquerade as ovarian cancer necessitating unnecessary

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laparoscopy[9-13] or can also mimic as an disseminated carcinomatosis. Ultrasonographic examination of the abdomen, pelvis and computerized tomography or magnetic resonance imaging of the case of abdominal/ pelvic tuberculosis presenting with a mass may differentiate it from ovarian malignancy[14] but the final diagnosis is reached by histology and serology[15].

CASE REPORTS

We present a series of five cases of recent past where diagnosis was a surprise.

CASES

Serial No.	Case history	Examination findings	Probable Diagnosis	Operative Interventions	Final Diagnosis
1.	40 years, with two live births presented with pain in abdomen, vomiting, loose stools since 15 days. Menstruation was two days with 30-60 days' interval with less flow.	Pale, normotensive, fullness in lower abdomen, vague supra-pubic cystic mass, margins not defined, cervix hypertrophied, eroded, first degree utero-cervical prolapse. On bimanual examination not possible to delineate uterus from cystic mass of 14 weeks' size of pregnant uterus, which seemed in continuity with cervix.	Clinical possibility of Uterine leiomyoma with Cystic Degeneration with Chronic Pelvic Inflammatory Disease or Ovarian mass or Ectopic pregnancy. USG showed left Tubo-ovarian mass. Endometrium obtained by D&C was in late secretory phase on histopathology.	On exploratory laparotomy, peritoneum was thickened , bowel loops were adherent to posterior wall of uterus, multiple biopsies, histopatholgy revealed tuberculosis everywhere including tubercular oophoritis.	Abdominal -Pelvic Tuberculosis.
2.	38 years, with three live births presented with something coming out of vagina, white discharge, itching over vulva and interrupted stream of urine since 16 yrs.	Pale, normotensive, abdomen- soft, nontender, third degree utero-cervical prolapse, cervix hypertrophied with erosion, cystocele, enterocele and rectocele. Bimanual examination- uterus six weeks' size of pregnant uterus, retroverted, retroflexed, mobile .	Third degree uterocervical prolapse with pelvic inflammatory disease.	During surgery for vaginal hysterectomy yellowish fluid came out from pelvic cavity. It turned out to be tubercular effusion.	Tubercular pelvic effusion.

3.	25 years, with two live births presented with lactational amenorrhea since 10 months, no menstrual abnormalities in past.	Pale, normotensive Abdomen had vague mass 7X4 cm, firm with ill defined margins in right iliac fossa. Bimanual, examination- uterus normal size retroverted, retroflexed, mass palpable in left fornix going to pelvic wall.	Clinical possibility Ovarian mass or Tubo-ovarian mass or Foreign Body Granuloma USG revealed heterogenous echotextured mass in right ovarian fossa with multiple glands about 4x2 cm, 2x1.5 cm.	USG guided FNAC of enlarged lymph nodes done, Cytology tubercular origin. AFB Culture positive.	Pelvic Tuberculosis.
4.	24 years, nullipara, married since two months, presented with pain abdomen since one month. Menstruation was infrequent with less duration and flow.	Pale, normotensive, Abdominal distension, Bimanual examination- uterus normal size with possible encysted fluid in pelvic cavity.	USG and CT abdomen showed bilateral enlarged ovaries with thick walled multiloculated cysts with ascites with diagnosis of ovary cytadenocarcinoma. Because of clinical suspicion of abdominal-pelvic Kochs D& C was done, endometrium was in proliferative phase, negative for AFB bacilli. Ascitic fluid was also negative.	With persisting diagnostic dilemma Exploratory laprotomy was done, omental umbrella was covering entire peritoneal cavity from transverse colon to fundus of uterus and broad ligament with bilateral encysted collection. Histopathology of tissues of peritoneum & omentum showed tubercular abscesses everywhere showing tubercular granuloma every where in pelvis.	Tubercular Granuloma with Tubercular abscesses.
5.	25 years , with two live births presented with sudden onset pain abdomen, vomiting & loose stools, since three days.	Pale,icteric, normotensive, abdomen distended, tender, ascites+, Bimanual examination showed cervix midposed , cervical erosion , bleeding from uterine cavity, uterus six weeks' size, right adnexa palpable, left side vague mass, adherent to broad ligament.	Ectopic pregnancy, clinically and by USG. Urine Pregnancy Test - Negative	Peritoneal fluid examination – no AFB . Exploratory laprotomy was done, bowel , uterus & tubes all were adherent with each other, Mesenteric lymph nodes were enlarged grossly.	Abdomen Pelvic Tuerculosis with Tubercular mesenteric lymphadenopathy

DISCUSSION

Recognition and understanding the spectrum of imaging features of extra-pulmonary tuberculosis can aid in the diagnosis of GT in the modern era .Before the HIV epidemic, approximately 15% of newly reported cases of tuberculosis had extra-pulmonary involvement [16], the number must be more now. Though tuberculosis can be diagnosed on FNAC by detection of acid fast bacilli (AFB) or caseating granulomas in smears[17] the only source of material generally available and studied from genital tract is endometrium, involved only in 50-60% of female GT and limit as the definite diagnosis. Further a single endometrial biopsy may miss the focal pathology. The present article gives a spectrum of clinical features and investigational need in patients presenting with varied symptoms and having GT even in present era. GT continues to be an important cause of infertility (both primary and secondary) and usually demonstrate multiple adhesions in the fallopian tubes, ovaries, bowel, omentum, and liver[2,18-20] . Laparoscopy is now a well-recognized procedure in the diagnosis of tuberculosis in infertile women, It can reveal presence of miliary granulomas, whitish yellow or opaque plaques surrounded by hyperemic areas over the fallopian tubes and uterus in acute stages. In chronic stages, the tubes show nodular salpingitis, patchy salpingitis, hydrosalpinx, caseosalpinx, or adhesions, as was observed in the present study. Surgery is usually not recommended but total abdominal hysterectomy with bilateral salpingo-oophorectomy may be required in women who do not desire continue to be symptomatic inspite of treatment or those not responding to medical treatment.

In an earlier study by the author [21], most of the patients were between 20 to 30 years of age, infertile but some were grand multipara. However the diagnosis came as a surprise in some cases where work up was being done with clinical diagnosis of leiomyoma or ectopic pregnancy or even malignant ovarian tumor. It is not possible to demonstrate *Mycobacterium tuberculosis* in every case. A number of newer investigations such as ELISA and polymerase chain reaction (PCR) have also been applied. In a case who presented with adnexal mass, ascites and raised CA-125 in a post menopausal woman, clinical features typically pointed diagnosis of ovarian malignancy. Other researchers also report that exploratory laparotomy should be considered when the diagnosis remains in doubt [22]. Around 20% women have tubo-ovarian masses and the diagnosis is made only after unnecessary laparotomy. Many patients may be completely asymptomatic, however, four major presenting complaints have been described with varying frequencies, infertility, abnormal bleeding, pelvic pain and menstrual abnormalities. Constitutional symptoms such as fever, sweat, anorexia and weight loss are not common. GT can not only mimic the presentation of ovarian malignancy but also cervical cancer. Studies reveal patients presented with infertility (65-70%) , pelvic/ abdominal pain (50-55%), and menstrual disturbances (20-25%) [23].

Genital TB is now undergoing a worrying recrudescence. We need to have an indepth knowledge of the pathology, the diagnostic means with which to discover it early and the correct therapeutic instruments to overcome. Research for early establishment of diagnosis, effective medical and surgical therapies with preservation of reproductive capability of the affected must continue.

REFERENCES

1. World Health Organisation, WHO report 2006. Global tuberculosis control: surveillance, planning, financing. WHO/HTM/TB/2006.362. Geneva, Switzerland:WHO, 2006.
2. Wise GJ, Marella VK. Genitourinary manifestations of tuberculosis. *Urol Clin North Am*. 2003; **30(1):**111-21.
3. Schaefer G. Female genital tuberculosis. *Clin Obstet Gynecol* 1976; **19(1):**223-39.
4. Bazaz Milk G, Maheshwari B, Lal N. Tuberculous endometritis: a clinico-pathological study of 1000 cases. *Br J Obstet Gynaecol* 1983; **90**: 84-6.
5. Tripathy SN. Laparoscopic Observation of pelvic organs in pulmonary tuberculosis. *Int J Gynecol Obstet* 1990; **32(2):**129-31.
6. Varma TR. Genital tuberculosis and subsequent fertility. *Int J Gynecol Obstect* 1991; **35(1)**:1-11.
7. Kim SH. Urogenital tuberculosis . In: Pollack HM, Mcclennan BL, DyerRB , Kenney PJ, *Clinical urography*; 2nd; Philedelphia, PA saunders, 2000: 1193-1228.

8. Chun SFS , stevens Dab- Infectious diseases. In; Wyangaarden JB,Smith LH Benett JC, editors. *Cecile Textbok of Medicine* . 19th ed, Philadelphia: WB saunders 1992; **2**:1903-5.
9. Penna L, Manyonda I, Amias A-Intra-abdominal miliary tuberculosis presenting as disseminated ovarian carcinoma with ascites and raised CA125. *Br J Obstet Gynaecol* 1993; **100(11)**: 1051-3.
10. Miranda P, Jacobs AJ, Roseff L. Pelvic tuberculosis presenting as an asymptomatic pelvic mass with rising serum CA-125 levels: a case report. *Reprod Med* 1996; **41**: 273-5.
11. Sheth SS. Elevated CA 125 in advanced abdominal or pelvic tuberculosis. *Int J Gynecol Obstet* 1996; **52**: 161-71.
12. Manidakis LG, Angelakis E, Sitakis S, Stefanaki P, Kalogeraki A, Manidaki A.Genital tuberculosis can present as disseminated ovarian carcinoma with ascites and raised Ca-125; a case report. *Gynecol Obstet Invest* 2001; **51(4)** :277-9.
13. Barutcu O, Erel HE, Saygili E, Yildirim T, Torun D. Abdominopelvic tuberculosis simulating disseminated ovarian carcinoma with elevated CA-125 level: report of two cases. *Abdominal Imaging* 2002; **27**: 465-70.
14. Bankier AA, Fleisehmann D, Wiesmayr MN, Putz D, Kontrus M, Hubsch P. Update: Abdominal tuberculosis-unusual findings on CT. *Clin Radiol* 1995; **50(4):** 223-8.
15. Tinelli Andreo, Malvasi Antonio, Vergara D. Martignago R, Nicolardi G, Tinelli R, Pellegrino M. Abdominopelvic tuberculosis in gynaecology: laproscopical and new laboratory findings. *Aust N Z J Obstet Gynecol* 2008 :**106** : 602-3.
16. Hopewell PC. A clinical view of tuberculosis. *Radiol Clin North Am* 1995; **33(4):** 641-53.
17. Sah SP, Bhadani PP, Regmi R , Tewari A, Raj GA .Fine needle aspiration cytology of tubercular epididymitis and epididymo-orchitis. *Acta Cytol* 2006; **50(3)**: 243-49.
18. Richards MJ, Angus D. Possible sexual transmission of genitourinary tuberculosis. *Int J Tuberc Lung Dis* 1998; **2(5)**: 439.
19. Sharma JB, Malhotra M, Arora R. Fitz-Hugh-Curtis syndrome as a result of genital tuberculosis: A report of three cases. *Acta Obstet Gynecol Scand* 2003; **82(3)**:295-7.
20. Gupta N ,Sharma J ,Mittal S,Singh N, Misra R, Kukreja M. Genital tuberculosis in Indian infertility patients. *Int J Gynaecol Obstet* 2007; **97(2)**: 135-8.
21. Chhabra S. Genital Tuberculosis – A Baffling Disease. *J Obst & Gyn of India* 1990; **40**: 569-73.
22. Jana N, Mukhopadhyay S, Dhali GK. Pelvic tuberculosis with elevated serum CA-125: a diagnostic dilemma. *J Obstet Gynae of India* 2007; **27**:226-7.
23. Mondal SK, Dutta TK. A ten-year clinicopathological study of female genital tuberculosis and impact of fertility. *J Nepal Med Assoc* 2009; **48**: 52.

Case Report

HYPERTROPHIC TUBERCULOSIS OF VULVA – A RARE PRESENTATION OF TUBERCULOSIS

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***Summary*:** The authors report a rare case of hypertrophic vulval tuberculosis of primary origin in a 26-year-old female patient. The diagnosis was mainly based on histopathological examination. Good outcome was obtained with antitubercular chemotherapy supplemented with surgical reduction for aesthetic concern. **[*Indian J Tuberc 2010; 57: 95-97*]**

Key words: Vulva, Tuberculosis, Female genital tract

INTRODUCTION

The incidence of female genital tract tuberculosis has declined since introduction of specific treatment, but it still represents a major problem in many third world countries, like India. Tuberculosis in females most frequently affects the upper genital tract, the fallopian tubes and endometrium and the vulvo-vagina is the rare site of the disease. Due to bizarre presentation, it is a challenge to the clinicians for diagnosis and proper management. Here, we present a rare case of hypertrophic vulval tuberculosis diagnosed on histopathology and successfully treated with antitubercular chemotherapy and surgery.

CASE REPORT

A 26-year-old nulliparous married woman from a rural background presented with vaginal discharge, painful ulcers on the right sided labia majora and swelling of vulva since last three years. Her menstrual cycle was regular with excessive blood loss. She received several courses of antibiotics before presenting to us. She denied any history of fever, cough or abdominal pain. On examination, she had hypertrophied vulva due to diffuse lymphoedema and elephantiasis, right side more than the left with a painful ulcer on the right sided labia (4x3cm) with serous discharge from it (Fig. 1). The clitoris was congested and very tender on touch. Internal examination was not possible due to pain. Right sided labia minora had multiple pigmented nodules. Both sided inguinal lymph nodes were discrete and non-tender. She was normoglycaemic with normal renal biochemical parameters. Erythrocyte Sedimentation Rate (ESR) was 48mm in first hour. Abdominal and pelvic ultrasonography and chest X-ray was non-contributory. Night blood for microfilaria was negative. Antibody tests for HIV and VDRL were negative. Mantoux test showed a strong reaction with 13mm erythema and induration. Smear taken from the ulcer did not show any AFB or Donovan bodies. Ultrasonography of lower abdomen suggested normal adenaxa and uterus.Biopsy from the ulcer edge of right sided vulva suggested sub-epidermal epithelioid granuloma with Langhan's giant cells (Fig. 2). Histopathology from lymph node showed reactive hyperplasia only. Tissue was not sent for AFB culture as histopathology suggested tuberculosis.

Her husband was examined and had no evidence for tubercular lesion in the epididymis, prostate or lung on clinical or radiological examination.

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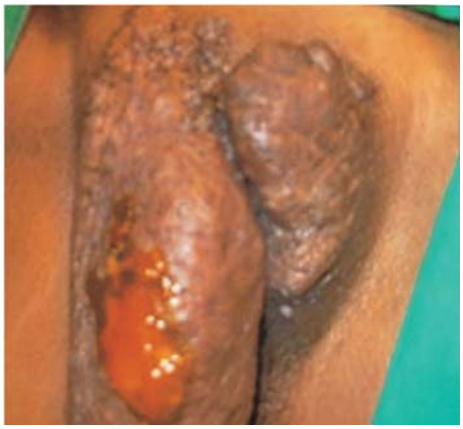

Fig. 1: Hypertrophied vulva with an ulcer on the right sided vulva.

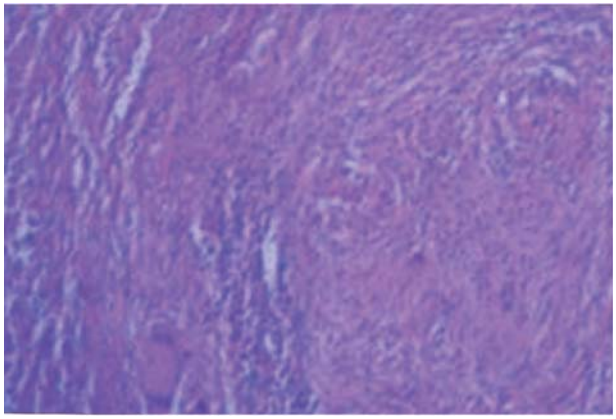

Fig. 2: Sub-epidermal epithelioid granuloma with Langhan's giant cells. (H & E X 400)

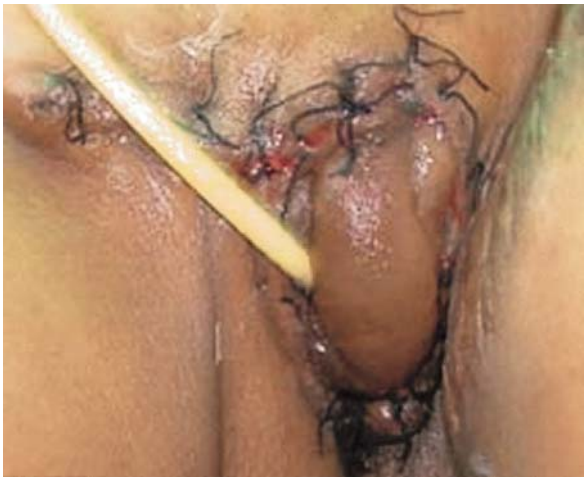

Fig. 3: Post-operative photograph on the fifth post-operative day.

After four weeks, the ulcer healed completely on antitubercular therapy but swelling regressed very little. The drugs used were INH, Rifampicin, Pyrazinamide and Ethambutol for two months and INH with Ethambutol for another seven months. As the patient was in active sexual life, a reconstructive surgery was undertaken with a good cosmetic appearance. There was oedema on the left side immediately on the post operative period (Fig. 3) which subsided within one month of surgery. The patient is on regular follow up for last one year without any recurrence.

DISCUSSION

Tuberculosis of vulva and vagina is very rare and found in only 1 to 2% of genital tract TB[1]. Tubercular lesions of vulva present as a small shallow ulcers[2], multiple sinus tracts[3] or rarely as elephantiasis of vulva[4]. A hypertrophic variety of vulval tuberculosis is the rarest form and mostly represents inflammatory induration and edema resulting from fibrosis and lymphatic obstruction[5]. It may arise by haematogenous spread, by direct extension from the lesions in the genital tract or exogenously from

sputum or sexual contact with a person harbouring epididymal or renal tuberculosis[4]. Usually, the presentations of symptomatic genital tract TB are infertility, abnormal vaginal bleeding, vaginal discharge, menstrual irregularities, abdominal pain or constitutional symptoms.

Though diagnosis of tuberculosis should be based on demonstration of AFB, but there is universal agreement that the bacilli are very rarely found in female genital tract even with flourescent techniques[4]. Most authors agree that histological examination is one of the most useful current means of establishing diagnosis of genital tract tuberculosis[3,4] as in this case. A strong suspicion of tuberculosis can lead to diagnosis.

We re-emphasize that tuberculosis should be kept in mind when a particular symptom fails to respond to empirical treatment. After chemotherapy, the residual deformity should be corrected by surgical excision for good cosmetic results, specially when the patient is sexually active.

REFERENCES

1. F. Akhalghi, A. B. Hamedi. Post menopausal tuberculosis of the cervix, vagina and vulva. *The Internet Journal of Gynecology and Obstetrics* 2004; **3(1).**
2. Chatterjee G, Kundu A, Das S. Vulval tuberculosis. *Indian J Dermatol* 1999; **44**:193-4.
3. Guruvare S, Kushtagi P. Genital tuberculosis manifesting as sinus tract. *The Internet Journal of Gynaecology and Obstetrics* 2007; **7(2).**
4. Agarwal J, Gupta JK. Female genital tuberculosis- A retrospective clinico-pathological study of 501 cases. *Ind J Pathol Microbiol* 1993; **36(4)**: 389-97.
5. Murugan S, Kaleeluliah MCA. Vulval tuberculosis with esthiomene. *Indian J Tuberc* 1988; **35**: 32-3.

Case Report

LUPUS VULGARIS WITH ENDOPTHALMITIS - A RARE MANIFESTATION OF EXTRAPULMONARY TUBERCULOSIS IN INDIA

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Summary: We report a case of 17-year-old girl who presented with gradual destruction of the nose along with endopthalmitis and loss of vision of the left eye. On nasal examination, left alae nasi and nasal cartilage was destroyed. Left eye showed signs of endopthalmitis with pthisis bulbi with complete loss of vision. Skin biopsy, FNAC of the lymph nodes were suggestive of tubercular etiology. However, patient did not have any evidence of pulmonary TB. We report this case due to the rare clinical features .The importance of a high index of suspicion and prompt treatment in such atypical forms to prevent morbidity cannot be over-emphasised. **[*Indian J Tuberc 2010; 57: 98-101*]**

***Key words*:** Lupus vulgaris, Endopthalmitis

INTRODUCTION

Although pulmonary tuberculosis (TB) is the most common manifestation of the disease, the bacilli can invade any organ resulting in extra pulmonary TB. Cutaneous TB forms an important domain of extra pulmonary form. The incidence of cutaneous TB is around 5.9 per 1000 population. Lupus vulgaris is the commonest morphological variant of skin TB and constitutes 74% of total cases.

The term "*Lupus*" was first coined by Erasmus Wilson in 1865, which compared the lesions to the ravages of wolf[1]. Lupus emphasizes the ulcerating and devouring character of the lesions. Although Lupus is not a rare entity in India, it is the bizarre clinical presentation and involvement of atypical sites, which often lead to inappropriate diagnosis causing significant morbidity. Thus it is vital for clinicians to have a high index of suspicion of such atypical forms.

The case below of lupus vulgaris of face causing extensive ulceration and disfigurement of the face with endopthalmitis is reported for its rarity in Indian population as compared to the western world.

CASE REPORT

A 17-year-old female patient from Goa (India) presented to OPD of Goa Medical College with chief complaints of nasal involvement since two years and deformity and loss of vision of left eye since one month. Nasal involvement started with painless red nodule over left nostril which gradually ulcerated over two years and led to destruction of the nose. Ocular complaints started with pustular lesion over the left lid margin which gradually caused purulent discharge, pain in the eye, loss of vision and considerable disfigurement. Patient also had noticed swelling on neck on left side since one month (Fig. 1).

There was no history of fever, cough, loss of appetite/ weight, difficulty in breathing/ swallowing, trauma, skin or mucosal lesions elsewhere in the body or history of recent travel. There was no past or family history of Kochs.

GENERAL EXAMINATION

Patient was a thinly built emaciated female (Fig. 2). Vitals were stable. No BCG

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scar was seen. Bilateral cervical lymph nodes were enlarged which were matted, firm in consistency and non-tender.

Detailed nasal examination

There was a visible nasal deformity with destruction of columella and membranous portion of the nasal septum. Left alae nasi was completely lost and showed an overlying necrotic ulcer of 4x3 cms with irregular margins and floor having purulent discharge. Thick reddish brown crusts were seen at places (Fig. 3).

Detailed ocular examination

There was periocular edema with upper eyelid showing ulceration and destruction. The cornea was perforated and underlying structures could not be evaluated due to pthisis bulbi. There was a thick purulent discharge from the eye with complete loss of vision. (Fig. 4).

INVESTIGATIONS

All biochemical and haematological investigations were within normal limits. Ocular

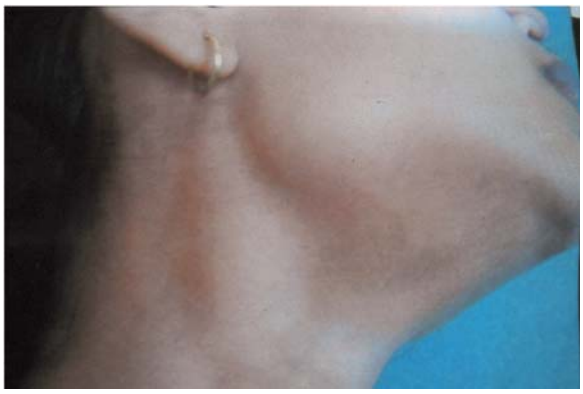


Fig. 1: Cervical lymphadenopathy

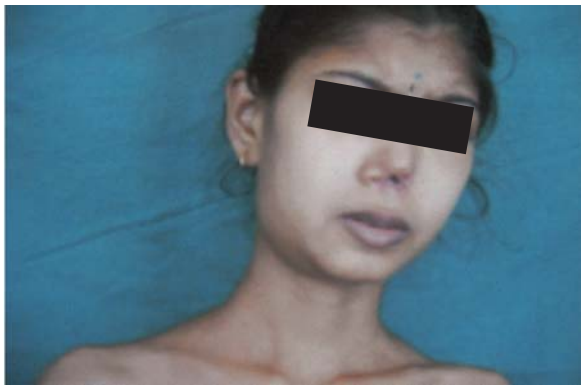


Fig. 2: General examination showing emaciation and disfigurement of the face

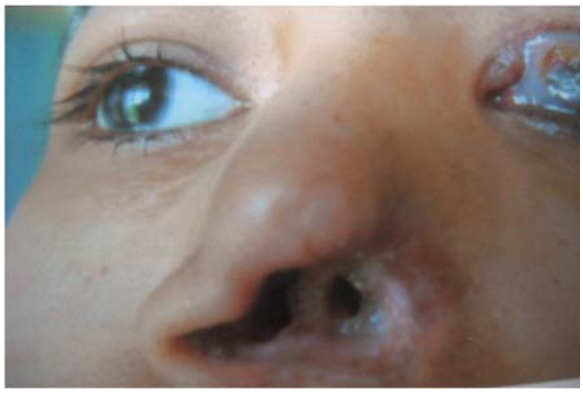


Fig. 3: Close-up view of destroyed alae nasi and nasal septum

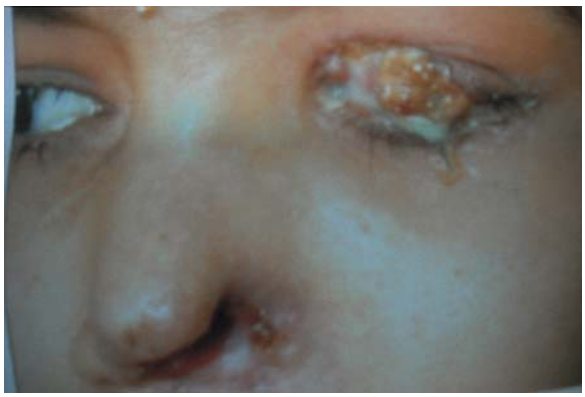


Fig. 4: Eye showing endophthalmitis and destruction of eye

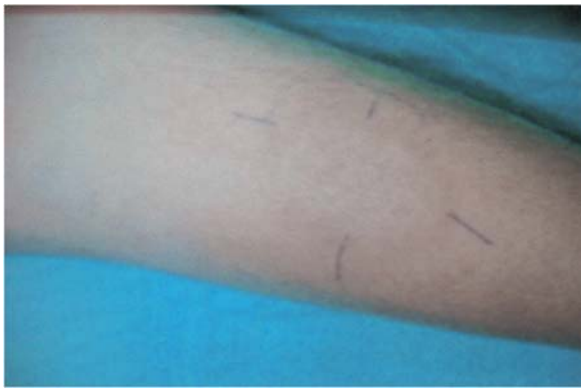


Fig. 5: Positive mantoux test

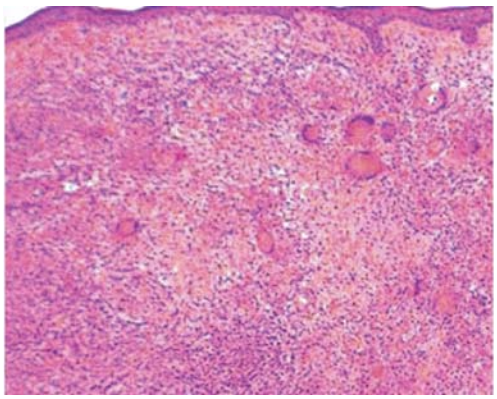


Fig. 6: Skin biopsy showing multiple epithelioid cell granulomas

swab was sterile. Chest radiograph revealed no abnormality. VDRL ,TPHA and ELISA for HIV were non-reactive. Sputum smears were examined using ZN staining which showed no acid fast bacilli. Mantoux test was strongly positive (Fig. 5).

FNAC of the cervical lymph nodes revealed caseation necrosis with lymphocytes and epithelioid cells. In Skin biopsy, epidermis was found to be atrophic and dermis contained multiple granulomas comprisig epithelioid cells and foreign body type of Langhan's cells with lymphocytic infiltrate. No AFB could be demonstrated in tissue sections (Fig. 6).

DISCUSSION

Lupus is a chronic, progressive post primary paucibacillary form of cutaneous TB occuring in individuals with moderate to high degree of immunity. Classically, it presents as a plaque, which shows apple jelly nodules on diascopy. Ulcerating and mutilating variant is a rare variant of lupus in which widespread ulcerations and scarring predominate. The deeper tissues and cartilage is invaded resulting in widespread contractures and deformities.

In European countries, over 80% of the lesions are situated on the face. However, in India trunk and the buttocks are the sites of predilection. Ocular manifestations of TB are infrequent and seen in 1.4% patients.[2]

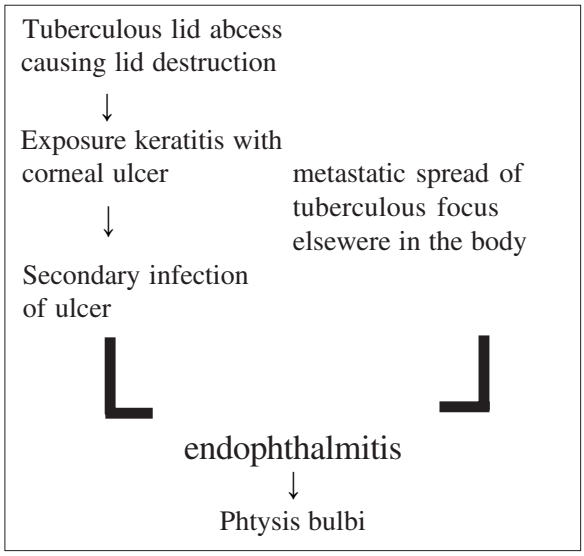
The rare features in our case can be highlighted as follows:-

Involvement of atypical site (face)
Presence of atypical variant (ulcerative and mutilating type)
Ocular manifestations in addition to skin involvement
Absence of pulmonary TB

After extensively reviewing the literature, we found very few case reports of such variant reported till date[3-6]. Over and above, none of these reports had any associated ocular findings. Thomas S *et al* (2005) reported a case of lupus similar to ours (except ocular involvement) causing destruction of the alae nasi, nasal septum, columella and termed it as "*Lupus Vorax*"[4].

Endopthalmitis is rarely associated with ocular TB. We presume that in our patient one

of the following factors might have been responsible:-



By the time our patient presented the eyeball was totally destroyed with pthisis bulbi starting to develop. Although the tubercular etiology was difficult to prove, with such an active cutaneous focus lying at close proximity, it is highly unlikely that the ocular findings were co-incidental.

Patient was started on DOTS (Category-1) following which active infection was controlled. However, the disfigurement persisted causing lowered self- esteem and significant morbidity. Hence early diagnosis and prompt treatment remains the cornerstone in management of such cases.

The incidence of TB, especially with the advent of HIV infection is on the rise, hence a clear knowledge and high index of suspicion regarding these forms is vital. Had our patient presented earlier, probably such a catastrophic disfigurement would have been arrested. Hence the importance of spreading awareness among general public about TB cannot be over-emphasized.

REFERENCES

1. Wolf K, Tappineir G. Mycobacterial Diseases : Tuberculosis and atypical mycobacterial infections. In: Fitzpatrick TB, Eiesen AZ, Wolf K (EDS) Mcgraw Hill, New York. 1987: 2152-70
2. Dinning WJ, Marston S. Cutaneous and ocular TB: A Review. *J R Soc Med* 1985; **78**: 576.
3. Kumar K ,Kuldeep CM, Mathur NR. Lupus vulgaris mimicking hansens diseases. *Indian J Derm* 1997; **42(2)**: 91-2
4. Thomas S, Suhas, K.M. Pai and A.R. Raghu. Report of a case with facial involvement. *BR Dent J* 2005; **198(3):** 135-7.
5. L Padmavati, L.Laksmanarao, M Ethirayan and B Krishnaswamy. Ulcerative lupus vulgaris of the face. An uncommon presentation in India. *Indian J Tuberc* 2007; **54**: 52-4.
6. Sanjay K. Rathi. Psoriasiform Lupus Vulgaris: An unusual presentation. *Indian J Derm* 2000; **45(4):** 201-02.

Case Report

TUBERCULAR BRAIN ABSCESS — CASE REPORT

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Summary*:** Central Nervous System (CNS) tuberculosis is a serious form of extra-pulmonary tuberculosis. CNS tuberculosis can present as meningitis, arachnoiditis, tuberculoma and brain abscess. Tubercular Brain Abscess (TBA) is a rare manifestation of central nervous system tuberculosis. With the advent of AIDS, more cases are being diagnosed, but very few have been reported in immunocompetent HIV negative patients. We present a case of TBA in a 23-year-old immunocompetent patient. The patient was given anti-tubercular treatment along with surgical excision. He showed significant improvement in all symptoms after weeks. ***[Indian J Tuberc 2010; 57:102-103]

Key words: Tuberculosis, Brain abscess

INTRODUCTION

Tuberculosis remains a major global problem and a public health issue of considerable magnitude.[1] TBA is a rare manifestation of intracranial tuberculosis , the usual presentation being tuberculoma or meningitis.[2,3] Though 57 cases of TBA were found in a review of world literature but only 18 cases fulfill the criteria laid down by Whitner *et al* in 1978.[4] In two series of cases from developing countries, TBA has been reported in 4% to 7.5% of patients with CNS tuberculosis without HIV infection compared to 20% in HIV positive patients.[5] Similar is the situation in India where tuberculosis is more common.[3]

The rarity and importance of identifying this entity prompted this case report. We are reporting a case of multiple TBA in a young immunocompetent adult.

CLINICAL REPORT

A 23-year-old man presented with two months' history of headache, diminished vision & diplopia of both eyes, giddiness and convulsions. He also gave history of fever off and on, vomiting, decreased appetite, generalized weakness and weight loss since three months. In the past, there is no history of contact with a patient of tuberculosis. Patient had no respiratory complaints and X-ray chest report was not suggestive of pulmonary Kochs.

He was admitted four months back for complaints of left sided headache, diminished vision of left eye, giddiness and convulsions. That time he was diagnosed as a case of left temporoparital abscess on CT scan and was operated for that. He was also put on Cat II anti-tubercular treatment (ATT). But patient himself has omitted ATT after four weeks.

He was thin built, conscious, well oriented with no sensorymotor deficiet except left sided diplopia. General and other systemic examinations were normal. All haematological and serum biochemical parameters, including liver function test (LFT) were normal. His fundus showed bilateral papilloedema with left lateral rectus paresis. The patient was HIV negative by ELISA using three different kits. Lumber puncture was done. CSF report showed protein 40 mg/dL, sugar 72 mg/dL, cell 107/mm3 with neutrophilic leucocytosis in peripheral blood smear. ESR was 18 mm at the end of one hour.

CT scan was done. It revealed multiple brain abscesses involving left temporo-parital, right

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temporal and left parieto-occipital region with bilateral sigmoid sinus thrombosis. CT scan report was not available as these pictures were with patient who later did not turn up for follow up.

By Borehole technique, under general anaesthesia, through right frontal previous incision, 70 ml pus was aspirated. A thick pus was sterile on aerobic and anaerobic culture. AFB were seen in direct smear examination and also were grown on culture within three weeks. Polymerase Chain Reaction (PCR) on pus showed presence of *Mycobacterium tuberculosis* specific DNA sequences using IS6110 primer.

Surgery was followed by antituberculous chemotherapy(Cat II). He showed significant improvement in all his symptoms.

DISCUSSION

Tubercular Brain Abscess represents an unusual expression of tuberculosis of CNS and probably is the result of an altered host response to invasion by tubercular bacilli. It is characterized by an encapsulated collection of pus, containing viable tubercle bacilli without evidence of tubercular granuloma. In 1978, Whitner proposed following criteria for establishing diagnosis of TBA[4]. A) Macroscopic evidence of a true abscess formation within the brain as confirmed during surgery or autopsy. B) Histological proof of presence of inflammatory cells in the abscess wall. C) Demonstration of AFB in the pus or abscess wall.

This condition is more commonly seen in immunocomprimised patients who were unable to mount a granulomatous inflammatory response. The incidence of TBA is on the rise with the advent of HIV infection.[1,5] Very few cases of TBA in immunocompetent individuals have been reported in literature.[2-4,6-10,12]

The pathogenesis of localized brain lesions is through haematogenous spread from a primary focus in the lung which is visible on chest radiograph in only 30% of cases.[1] Chattopadhyay P reported TBA in a young patient without previous history of TB.[12] He diagnosed a case of TBA by PCR. In the case reported here, patient had no respiratory complaints. The diagnosis of TBA is made mainly on the basis of demonstration of AFB in pus by culture (Gold standard) and absence of growth of other pathogenic organisms and also by PCR. Our case is typical and satisfies all the criteria of a true TBA.

The failure to recognize the tubercular aetiology of abscesses could result in uncontrolled spread of infection and death. Patient in our case did well after surgery and ATT. He might be called as a case of defaulter as per case definition by RNTCP (Revised National Tuberculosis Control Programme)[11]. He showed improvement at the time of discharge but later could not be followed up as he did not come.

REFERENCES

1. Ravinder Kumar Garg. Tuberculosis of the CNS. *Postgrad Med J* 1999; **75**: 133-40.
2. M. L. Babu Shavinder. Tubercular brain abscess. *JK-Practitioner* 2002; **9(4):** 262-3.
3. S. C. Tandon, S. Asthana, S. Mohanty. Unusual presentation of Tubercular brain abscess. *Indian J Tuberc* 1992; **39**: 41-3.
4. Whitner DR. Tuberculosis brain abscess. Report of a case and review of literature. *Archives of Neurology* 1978 ; **35(3)**: 148-53.
5. Farrar DJ Flanigan TP, Gordon NM, Gold RL *et al*. Tuberculous brain abscess in a patient with HIV infection: case report and review. *Am J Med* 1997; **102** : 297-301.
6. K. Vasudeva Devadiga, A. Date, K.V. Mathai *et al*. Tuberculous abscess of the brain. *Neurology India* 1969; **17**: 35-7.
7. Chandramukhi A, Rao T.V., Rao A.N.S. Tuberculous brain abscess. Report of two cases. *Neurology India* 1981; **29**: 38.
8. Sharma V, Newton G. Multiple tuberculous brain abscesses. *Indian J Tuberc* 1992; **39**: 185-8.
9. Rajeshwari Ramchandran, Ranjani Ramchandran, S. Kalyanaraman, R. Prabhakar. A rare presentation of miliary Tuberculosis. *Indian J Tuberc* 1993; **40**: 205.
10. Amonkar GP, Jashnani KD, Deshpande JR. Tuberculous brain abscesses of the brain. *Bombay Hosp J* 2004; **45(1)**: 86-8
11. RNTCP-an overview. A manual for sensitization of medical college faculty. Published by Maharashtra State Tuberculosis Control Society, Mumbai 2004.
12. Chattopadhyay P and Kundy AK. Tuberculosis of brain abscess. A diagnostic and therapeutic challenge. Letter to the Editor. *JAPI* 2006; **54**: 829.

Case Reports

CO-EXISTING TUBERCULAR AXILLARY LYMPHADENITIS WITH CARCINOMA BREAST CAN FALSELY OVER-STAGE THE DISEASE - CASE SERIES

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Summary: The synchronous occurrence of tuberculosis and carcinoma in breast is unusual. The simultaneous occurrence of both the diseases can complicate the neoplastic disease. The diagnosis and treatment of tuberculosis in a patient with cancer assumes importance as it can prevent high mortality in patients with co-existent disease and thereby create problems in treatment decision. Axillary lymph node enlargement in breast cancer patient is not always caused by metastatic tumour of the breast even in the ipsilateral axillary nodes. We present here six case reports as an example of tuberculous axillary lymphadenitis co-existing with invasive ductal carcinoma of the breast. Accurate diagnosis has helped in down-staging carcinoma of the breast and also in identifying curable disease. ***[Indian J Tuberc 2010; 57:104-107]***

Key words: Axillary tubercular lymphadenitis, Carcinoma breast.

INTRODUCTION

Extrapulmonary Tuberculosis (TB) constitutes about 15 to 20 per cent of all cases of tuberculosis with lymph nodes being the most common site of involvement.[1] Co-existing TB with carcinoma has been previously reported in most organs, especially with lung cancer.[2] The synchronous occurrence of tuberculosis and carcinoma in breast is unusual and the literature includes single case reports[3-9] probably due to the declining incidence of TB in the West. The simultaneous occurrence of both the diseases in breast can complicate the neoplastic disease and thereby create problems in treatment decision. However, there is a paucity of data in this aspect, so we decided to analyze the data available in Sri Aurobindo Institute of Medical Sciences (SAIMS) and present here six case reports. All the cases show co-existing carcinoma breast with TB axillary lymphnode leading to a false overstaging of the disease and consequently the patients lost the opportunity of a conservative surgery.

CASE REPORTS

A total of 109 consecutive cases of carcinoma breast who underwent Modified Radical Mastectomy (MRM) in SAIMS from January 2007 to June 2008 were studied. Out of these, six cases were picked up and found to be having co-existing carcinoma breast and TB axillary lymphnode, each case is presented with its individual details. All the cases are summarized (Table).

Case 1

A 50-year-old female underwent MRM in SAIMS in February 2007 for a lump right breast after initial diagnosis of carcinoma breast. On investigations, she had multiple palpable axillary lymph nodes on the same side, few were fixed, and was clinically staged as T2N2. Histopathological examination of the MRM specimen revealed a tumour of size 3.5x3x2.5cm showing features of Infiltrating Duct Carcinoma (IDC) (Fig. 1). From the 21 axillary lymph nodes studied, seven showed

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Table: Details of the six cases summarized

Case no.	Age (yrs.)	Clinical stage	Surgery	Diagnosis	Axillary node Metastasis	Axillary Node Tuberculosis	Path Stage
1	50	T2 N2	MRM	IDC	0 out of 21	Present (in 7 nodes)	T2 No
2	48	T1 N1	MRM	IDC	0 out of 18	Present (in 2 nodes)	T1 No
3	45	T2 N2	MRM	IDC	0 out of 14	Present (in14 nodes)	T2 No
4	75	T4 N2	MRM	IDC	2 out of 12	Present (in10 nodes)	T4 N1
5	60	T3 N2	MRM	Medullary	8 out of 15	Present (in 4 nodes)	T3 N2
6	35	Tx N2*	MRM	IDC	0 out of 21	Present (in 21 nodes)	Tx No

MRM: Modified Radical Mastectomy , IDC : Infiltrating Duct Carcinoma, *Post lumpectomy case

features of TB with caseating granulomas (Fig. 2) and none showed any evidence of metastasis. The pathological staging finally reported was T2N0.

Case 2

A 48-year-old female presented with a lump in left breast in August 2007 in the surgical Out Patient Department (OPD) of SAIMS and a few palpable axillary nodes. Preliminary investigations confirmed the diagnosis as carcinoma breast with clinical staging as T1N1. She underwent MRM and the pathological examination of the specimen showed a tumour size 2x2x1.5 with features of IDC, 18 axillary nodes were studied, none showed evidence of malignancy and two nodes showed TB. The patient was finally staged as T1N0.

Case 3

In October 2007, a 45-year-old female attending SAIMS OPD was found to have a right breast lump on mammography. Clinical examination showed multiple fixed axillary nodes on the same side. She underwent MRM with a diagnosis of breast malignancy, clinically staged as T2N2. The specimen showed a tumour of size 3.4x3x2cm, diagnosed as IDC on histopathology. Of the 14 axillary nodes studied, all showed features of caseating TB and none showed metastastic disease. The pathological staging of the patient was T2N0.

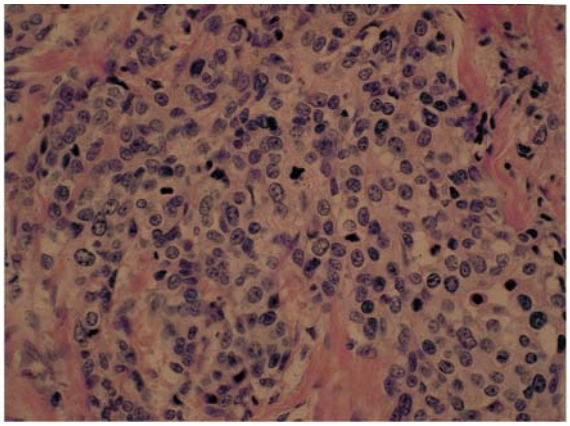

Fig. 1: Infiltrating duct carcinoma breast, H and E X400

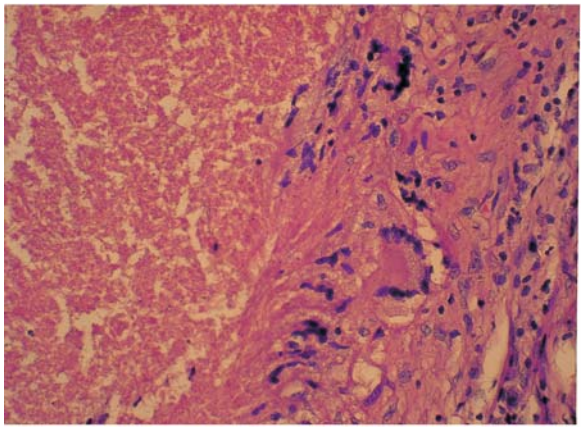

Fig. 2: Classical caseating tubercular granuloma in an axillary lymph node. H and E X400

Case 4

A 75-year-old female presented with complaints of lump right breast in January 2008. On examination, she had a lump of size 4x3cm with ulceration of the overlying skin, axillary lymph nodes were enlarged and fixed, she was clinically diagnosed as carcinoma breast, stage T4N2 and underwent MRM. The examination of the specimens showed a tumour measuring 3.5x3x2.5cm, IDC, involving the skin with pagetoid change. Twelve axillary nodes were studied out of which two showed metastasis and rest 10 showed TB. None of the nodes showed combined malignancy and TB. A pathological staging of T4N1 was given.

Case 5

In January 2008 a 60-year-old female underwent MRM for carcinoma right breast with enlarged axillary nodes. Pathological examination of the specimen revealed a tumour size of 5.5x4x3.5cm showing features of medullary carcinoma. Of the 15 axillary nodes, eight showed metastasis and four nodes showed caseating TB. None of the lymph nodes studied showed co-existing TB and metastasis in the same node. Patient was staged as T3N2.

Case 6

A 35-year-old presented in SAIMS in May 2008 with a history of lumpectomy elsewhere 15 days back which was diagnosed as IDC. She had multiple fixed axillary nodes and underwent MRM at SAIMS. The specimen showed a lumpectomy cavity of 5x4cm, histopathology showed residual disease in the cavity wall. All the 21 axillary nodes showed TB, none showing evidence of malignancy.

None of these cases had primary mammary or pulmonary tuberculosis. All the above six cases received chemotherapy with local radiation followed by a complete course of ATT. They are being followed till date and show no evidence of disease.

DISCUSSION

The association of tuberculosis and cancer has been recorded in most of the organs and has been described and explained by many authors in many diverse ways. Kaplan *et al* reviewed 58,245 patients with cancer and identified 201 cases of coexisting tuberculosis.[2] Highest prevalence was seen in patients with Hodgkin's disease (96/10,000 cases) followed by lung cancer (92/10,000), lymphosarcoma (88/10,000) and reticulum cell sarcoma (78/10,000). Among 14,742 cases of breast reviewed by them, only 28 had co-existing tuberculosis in breast, a prevalence of 19/10,000.

The clinical situations that arise are the presence of carcinoma and tuberculous mastitis, carcinoma in the breast with axillary tuberculous adenitis or both.[9] All the six cases in our study had carcinoma breast with axillary tuberculous adenitis without any primary mammary or pulmonary TB.

Co-existence of two diseases in one organ is always a diagnostic and therapeutic challenge.[10] This can create a dilemma in diagnosis and treatment as there are no pathognomonic symptoms or signs to distinguish both diseases. Most decisions in the management of breast cancer are taken based on TNM staging of the tumours. While both carcinoma of the breast and tuberculosis (TB) are common in developing countries, their co-existence in the breast is rare which can lead to overestimation of the tumour size, and therefore, these patients lose the opportunity for breast conservation due to this.[9] Presence of palpable axillary nodes, which may be due to tuberculous lymphadenitis, also leads to the overstaging of nodes. Treatment compliance, which is a major problem in developing countries, may be a problem when two major diseases are being treated. In the above set of patients discussed, the synchronous presence of axillary TB led to clinical overstaging of malignancy based on which the management decisions were taken.

CONCLUSION

The co-existence of TB and carcinoma requires a high index of suspicion for diagnosis, concomitant treatment of both diseases, and counselling of patients to ensure compliance. Axillary lymph node enlargement in breast cancer patients is not always metastatic disease. We have described six cases of co-existence of carcinoma in breast

and an ipsilateral enlargement of axillary lymph nodes caused by tuberculosis. Accurate diagnosis has helped us in down-staging the disease and also identifying curable disease which helped in modifying the treatment protocol. **The diagnosis and treatment of tuberculosis in a patient with cancer assumes importance as it can prevent high mortality in patients with co-existent disease.**

REFERENCES

1. Sharma SK, Mohan A. Extrapulmonary tuberculosis. A review article. *Indian J Med Res* 2004; **120**: 316-53.
2. Kaplan MH, Armstrong D, Rosen P. Tuberculosis complicating neoplastic diseases: a review of 201 cases. *Cancer* 1974; **33**: 850-8.
3. Singh A and Saxena S. Infiltrating ductal carcinoma of the breast, metastatic to axillary lymph nodes harbouring primary tuberculous lymphadenitis. *Pathology & Oncology Research* 2006; **12**:188-9.
4. Balasubramanian SP, Rao MP, Jayaram S, Bose SM. Co-existent mammary tuberculosis and malignant disease. *Can J Surg* 2001; **44**: 224–5.
5. Pandey M, Abraham EK, Chandramohan K, Rajan B. Tuberculosis and metastatic carcinoma co-existence in the axillary lymph node: a case report. *World J Surg Oncol* 2003; **1**: 3.
6. Fujii T Kimura M, Yanagita y, Koida T, Kuwano H. Tuberculosis of axillary lymph nodes with primary breast cancer. *Breast Cancer* 2003; **10**: 175–8.
7. Robinson AJ, Horne CA, Weaver A. Co-existence of axillary tuberculous lymphadenitis with lymph node metastasis from a breast carcinoma. *Clin Oncol (R Coll Radiol)* 2001; **13**:144.
8. Chottanapund S, Wongtawatchai P. Tuberculous axillary lymphadenitis co-existence in patients with invasive carcinoma of the breast: A case report. *The THAI journal of surgery* 2004; **25**:121-24.
9. Tulasi N, Raju P, Damodaran V, Radhika T: A spectrum of co-existent tuberculosis and carcinoma in the breast and axillary lymph nodes: Report of five cases. *The breast* 2006; 15:437-39
10. Khurram M, Tariq M and Shahid P. Breast cancer with associated granulomatous axillary lymphadenitis: A diagnostic and clinical dilemma in regions with high prevalence of tuberculosis. *Pathology - Research and Practice* 2007; **203**: 699-704.

Short Communication

SIGNIFICANT REDUCTION OF GRANULOMAS IN Nrf2-DEFICIENT MICE INFECTED WITH *MYCOBACTERIUM TUBERCULOSIS*

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Summary

***Objective*:** We have reported previously that mice deficient in nuclear erythroid 2 p45-related factor 2 (Nrf2), which regulates the expression of antioxidant and detoxification genes, showed significant susceptibility to airway inflammatory responses when exposed to diesel exhaust particles for eight weeks[2]. As disruption of Nrf2 promotes immune cells that stimulate Th2-like immunoresponsiveness, Nrf2-deficient mice may be resistant to *M. tuberculosis* infection.

Setting: Nrf2-deficient mice were infected with *M. tuberculosis* aerially, and the size of their granulomas and cytokine mRNA expression were compared with those of wild-type mice.

***Results*:** Significant reduction of granuloma formation and tubercle bacilli in granulomas was noted in the deficient mice 27 weeks after infection, concurrently with higher expression of IL-2 and IL-13 mRNA.

***Conclusion*:** It is concluded that Nrf2 inversely regulates *M. tuberculosis*-induced granuloma development at the late stage.

[Indian J Tuberc 2010; 57:108-113]

***Key words*:** Nrf2, C57BL/6 mouse, *M. tuberculosis*, Th2 cytokine

INTRODUCTION

Nuclear erythroid 2 p45-related factor (Nrf2) is a redox-sensitive transcription factor that regulates the expression of antioxidant and detoxification genes[3]. Disruption of Nrf2 in mice enhances susceptibility to severe airway inflammation and asthma and to airway inflammatory responses induced by low-dose diesel exhaust particles[2,4]. Moreover, its disruption promotes dendritic cells that stimulate Th2-like immunoresponsiveness upon activation by ambient particulate matter [7]. Therefore, it is thought that Nrf2-deficient mice are resistant to mycobacterial infection. These previous findings prompted us to explore further the role of Nrf2 in murine tuberculosis.

MATERIAL AND METHODS

Nrf2-deficient C57BL/c mice were generated as described previously[1]. The Nrf2-deficient and wild-type (WT) mice were infected by placing them into the exposure chamber of an aerosol generator (Glas-Col, Inc., Terre Haute, Ind.) in which the nebulizer compartment was filled with 5 ml of a suspension containing $3x10^6$ CFU of Kurono tubercle bacilli (ATCC35812) under conditions that would introduce about 500 bacteria into the lungs of each animal[6,8]. At 1, 3, 5, 7, 12, 27 and 30 weeks after aerosol infection, the lungs, spleens, and livers were excised for histologic examination and the right lobes of the lungs and some spleen tissues were excised for CFU assay.

RESULTS

Fig. 1 shows the representative lung histology at 12 (A and B) and 27 (C and D) weeks after infection. The granulomas looked similar in both Nrf2-deficient and WT mice, lacking central necrosis and consisting of lymphocytes, epithelioid macrophages and foamy macrophages. We measured the sizes of 30 pulmonary granulomas in these mice. There was no significant difference in the size of granulomas between Nrf2-deficient and WT mice at 12 weeks after infection (475±35 ìm vs. 493±40 ìm). However, a significant granuloma size difference was observed at 27 weeks after

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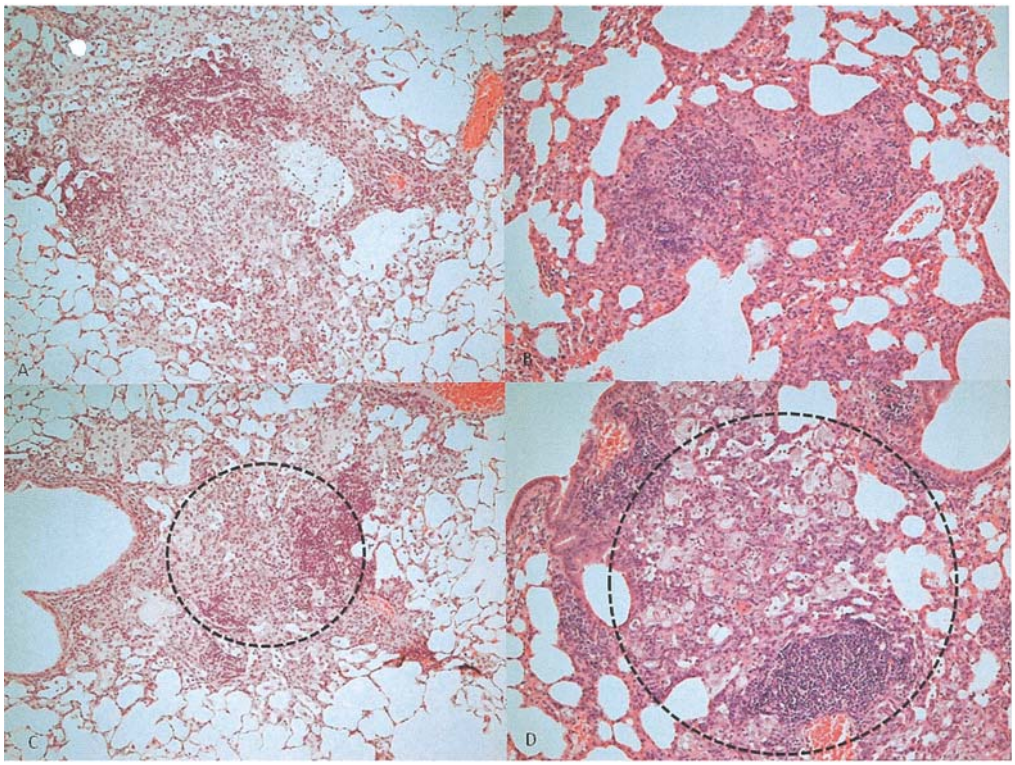

Fig. 1. Histologic examination of lung tissues. The mice were sacrificed 12 and 27 weeks after airborne infection with *M. tuberculosis* Kurono strain. Hematoxylin and eosin stain. x100. (A) Pulmonary tissue from a Nrf2-deficient mouse 12 weeks after infection with the Kurono strain. (B) Pulmonary tissue from a WT mouse 12 weeks after infection. (C) Pulmonary tissue from a Nrf2-deficient mouse 27 weeks after infection (513±45 ìm). (D) Pulmonary tissue from a WT mouse 27 weeks after infection (1,755±120 ìm). Note the sizes of the granulomas (Ì%) in C and D.

infection (513±45 ìm vs. 1,755±120 ìm)($p<0.05$). Next, we counted CFU in the infected lung and spleen tissues. The tissues were homogenized with a mortar and pestle, and then 100 ìl of the homogenate was plated in 10-fold serial dilutions on 1% Ogawa slant media. Colonies on the media were counted after a 4-week incubation at 37°C[8].

Fig. 2 shows CFU in the lung and spleen tissues of Nrf2-deficient and WT mice (6 mice each) infected with *M. tuberculosis*. At one week after infection, tubercle bacilli were recovered only from lung tissues. However, by three weeks after infection, the mycobacterial load in the organs had increased with a similar pattern in the mice. Surprisingly, at 27 weeks after infection, Nrf2-deficient mice showed 10-fold more tubercle bacilli in the lungs than did WT mice ($p<0.05$).

Part of the left lung was subjected to reverse transcriptase PCR (RT-PCR) analysis to examine the expression levels of IFN-ã, TNF-á, IL-1â, IL-2, IL-4, IL-10, IL-12, IL-13, and iNOS mRNAs. ABI Taqman Gene Expression Assay and an ABI Prism 7900HT Sequence Detection System (Applied Biosystems Inc.) were used for relative quantitative measurement of the mRNA expression. The results were expressed as relative expression quantities of the targets in comparison with those of non-infected mice that were calibrated against the expression of an internal control gene, glyceraldehyde-3-phosphate dehydrogenase

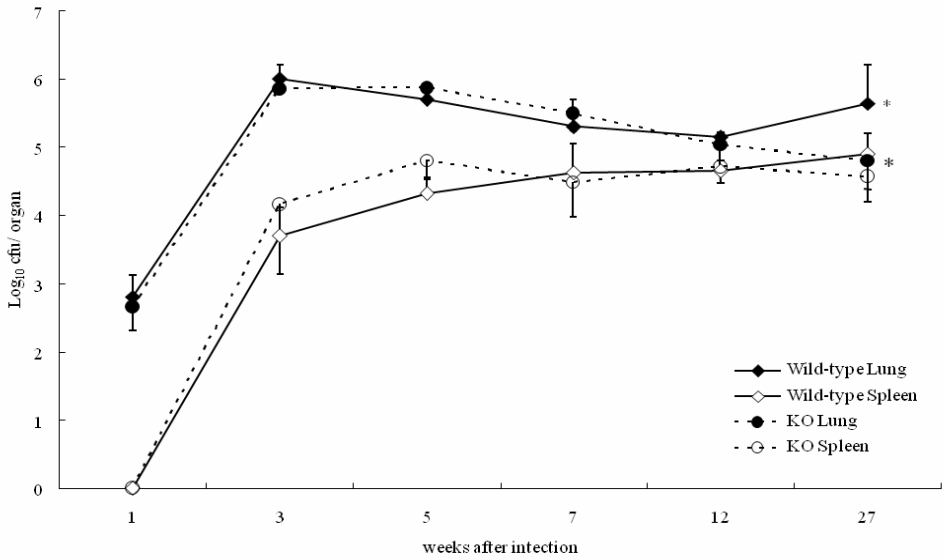

Fig. 2: CFU in lung and spleen tissues of Nrf2-deficient and WT mice (6 mice each) exposed to the Kurono strain by the airborne route. *p<0.05 Nrf2-deficient mice vs. WT mice.

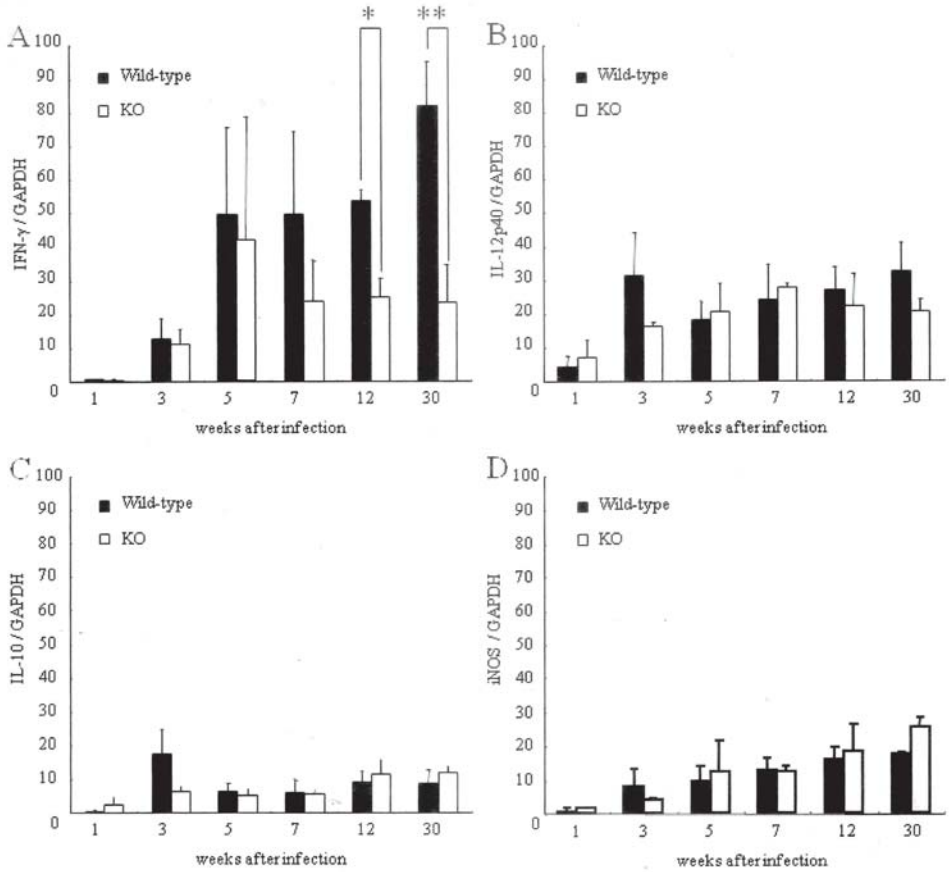

Fig. 3A: Real-time RT-PCR for genes of various cytokines and iNOS in the lungs. *p<0.05 Nrf2-deficient mice vs. WT mice.

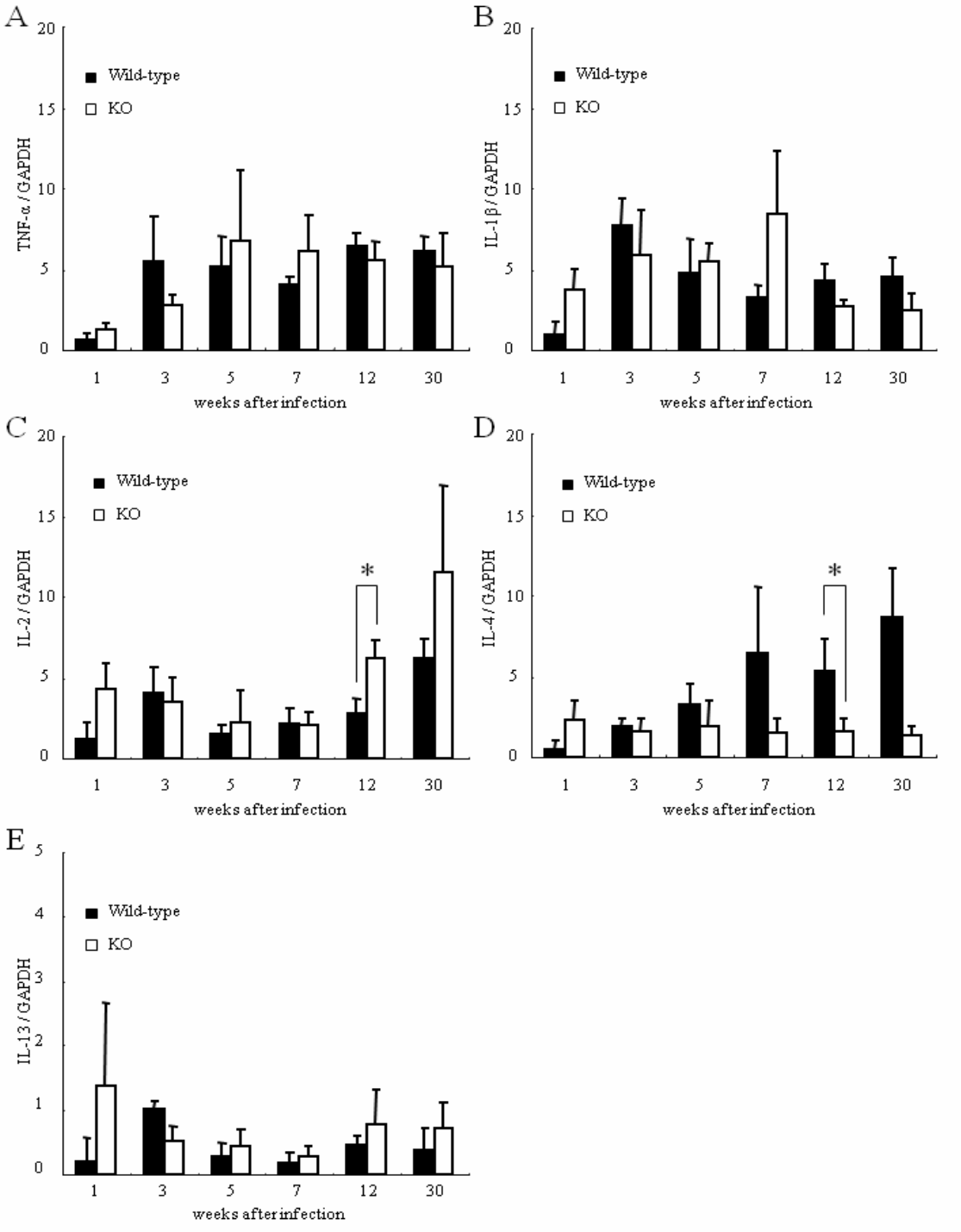

Fig. 3B: Real-time RT-PCR for genes of various cytokines and iNOS in the lungs. *p<0.05 Nrf2-deficient mice vs. WT mice.

(GAPDH)[2,9,10]. The expression of pulmonary IFN-ã mRNA was lower at every indicated time point in Nrf2-deficient mice, and there was a significant difference in the expression level between Nrf2-deficient and WT mice at 12 and 30 weeks after infection ($p<0.05$). Expression of pulmonary TNF-á, IL-1â, IL-10, IL-12, and iNOS mRNA was observed at every time point in both mouse groups, but no significant difference was recognized. However, there was a significant difference in IL-2 mRNA expression between Nrf2-deficient and WT mice 12 weeks after infection ($p<0.05$). Expression of IL-13 mRNA was higher in Nrf2-deficient mice than in WT mice at every time point, but there was no significant difference between the groups. (Figs. 3a and 3b).

DISCUSSION

As indicated by other reports, disruption of Nrf2 in mice enhances susceptibility to asthma and airway inflammatory responses induced by inhalation of diesel exhaust particles because Nrf2-deficient mice have increased Th2 cytokine responses in the absence of functional Nrf2[2,4]. As long as bronchoalveolar fluid of Nrf2-deficient mice exposed to low-dose diesel exhaust particles for eight weeks is used, IL-12 and IL-13 secretion levels are significantly higher than those in WT mice [2]. In our present study, thIL-13 mRNA expression level was higher than in WT mice, but no such tendency was observed for IL-12 expression. This may reflect the difference in the experimental design (mycobacterial infection vs. diesel exhaust exposure). It is difficult to explain exactly why the sizes of granulomas decreased at the late stage (in this case, 27 weeks after infection) of mycobacterial infection. Although Nrf2-deficient mice are Th2-directed immunologically, Th1-related cytokines such as IFN-ã and IL-2 remain intact functionally. Also, several genes of antioxidants in the lungs including glutamate-cysteine ligase, glucose-6-phosphate dehydrogenase, glutathione-S-transferase, heme-oxygenase-1, superoxide dismutase 2 and glutathione reductase are not sufficiently induced in Nrf2-deficient mice[2]. Further study will be required to elucidate this issue.

It is intriguing that Nrf2, which regulates the expression of antioxidant and detoxification genes and confers cytoprotection against oxidative stress and apoptosis in normal cells, is closely related to promotion of granuloma growth. As RNAi-mediated silencing of Nrf2 gene expression in lung cancer inhibits tumour growth, mycobacterial granulomas and tumours may reveal similar biological behaviour[5].

Thus, targeting of Nrf2 activity in mycobacterial granulomas could be a promising strategy for inhibiting granuloma growth. In summary, disruption of Nrf2 results in reduction of granuloma growth at the late stage and a decrease of pulmonary CFU. This is the first report to indicate a link between mycobacterial infection and oxidative stress.

REFERENCES

1. Itoh, K., T. Chiba, S. Takahashi, T. Ishii, K. Igarashi, Y. Katoh, T. Oyake, N. Hayashi, K. Satoh, I. Hatayama, M. Yamamoto, and Y. Nabeshima. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem. Biophys. *Res Commun* 1997; **236**: 313-22.
2. Li, Y., H. Takizawa, A. Azuma, T. Kohyama, Y. Yamauchi, S. Takahashi, M. Yamamoto, T. Kawada, S. Kudoh, and I. Sugawara. Disruption of Nrf2 enhances susceptibility to airway inflammatory responses induced by low-dose diesel exhaust particles in mice. *Clin Immunol* 2008; **128:** 366-73.
3. Nguyen, T., P. J. Sherratt, and C. B. Pickett. Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. *Annu Rev Pharmacol Toxicol* 2003; **43:** 233-60.
4. Rangasamy, T., J. Guo, W. A. Mitzner, J. Roman, A. Singh, A. D. Fryer, M. Yamamoto, T. W. Kensler, R. M. Tuder, S. N. Georas, and S. Biswal. Disruption of Nrf2 enhances susceptibility to severe airway inflammation and asthma in mice. *J Exp Med* 2005; **202**: 47-59.
5. Singh, A., S. Boldin-Adamsky, R. K. Thimmulappa, S. K. Rath, H. Ashush, J. Coulter, A. Blackford, S. N. Goodman, F. Bunz, W. H. Watson, E. Gabrielson, E. Feinstein, and S. Biswal. RNAi-mediated silencing of nuclear factor erythroid-2-related factor 2 gene expression in non-small cell lung cancer inhibits tumor growth and increases efficacy of chemotherapy. *Cancer Res* 2008; **68**: 7975-84.
6. Sugawara, I., and S. Mizuno. Higher susceptibility of type 1 diabetic rats to *Mycobacterium tuberculosis* infection. *Tohoku J Exp Med* 2008; **216**: 363-70.
7. Williams, M. A., T. Rangasamy, S. M. Bauer, S. Killedar, M. Karp, T. W. Kensler, M. Yamamoto, P. Breysse, S. Biswal, and S. N. Georas. Disruption of the transcription factor Nrf2 promotes pro-oxidative dendritic cells that

stimulate Th2-like immunoresponsiveness upon activation by ambient particulate matter. *J Immunol* 2008; **181**: 4545-59.
8. Yamada, H., S. Mizuno, M. Reza-Gholizadeh, and I. Sugawara. Relative importance of NF-êB p50 in mycobacterial infection. *Infect Immun* 2001; **69**: 7100-05.
9. Yamada, H., T. Udagawa, S. Mizuno, K. Hiramatsu, and I. Sugawara. Newly designed primer sets available for evaluating various cytokines and iNOS mRNA expression in guinea pig lung tissues by RT-PCR. *Exp Anim* 2005; **54**: 163-72.
10. Yamada, H., S. Mizuno, A. C. Ross, and I. Sugawara. Retinoic acid therapy attenuates the severity of tuberculosis while altering lymphocyte and macrophage numbers and cytokine expression in rats infected with *Mycobacterium tuberculosis*. *J Nutr* 2007l; **137**: 2696-700.

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I, Secretary General of the Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110 001, hereby declare that the particulars given above are true to the best of my knowledge and belief.

Secretary General
On behalf of the Tuberculosis Association of India

Short Communication

PREVALENCE OF PULMONARY TUBERCULOSIS AMONGST THE BAIGAS - A PRIMITIVE TRIBE OF MADHYA PRADESH, CENTRAL INDIA

R. Yadav[1], V. G. Rao [1], J. Bhat[1], P.G. Gopi[2], N. Selvakumar [2] and D.F. Wares[3]

(Received on 2.9,2009; Accepted on 23.2.2010)

Summary

Background: A community-based cross-sectional tuberculosis (TB) disease prevalence survey was undertaken amongst the Baiga primitive tribal community of Baiga Chak in central India.

Material and Methods: A population of 2,359 was covered under the study. Sputum samples were collected from chest symptomatics and examined for smear microscopy and culture.

Results: Overall prevalence of PTB was 146 (95% C.I: 0 - 318) per 100,000 population.

Conclusion: The findings suggest that TB is not a major public health problem amongst this tribal group. However, there is still the need to maintain and further strengthen TB control measures on a sustained and long term basis in the area.

[Indian J Tuberc 2010; 57:114-116]

Key words: Pulmonary Tuberculosis, Tribal, Baiga, Central India

INTRODUCTION

Tuberculosis (TB) remains a major global public health problem and its control a challenge in developing countries like India. Baseline data on the tuberculosis (TB) situation is essential to know the extent of the problem and also to know the impact of the control programme in the popultion. A nation-wide disease survey conducted by the Indian Council of Medical Research (ICMR) during 1955-58 provided, for the first time, information on the TB disease situation in the general population of the country.[1] The survey, however, did not assess the TB disease situation among tribal population in the country. Few studies have been conducted in tribal populations since the ICMR survey.

Tribal populations are groups of people sharing common cultural and socio-religious beliefs, residing in a particular geographic area and often practising endogamy. They are an underprivileged group usually having poor access to health delivery systems. Among the tribal groups in the state of Madhya Pradesh, three groups have been identified as "primitive" based on their low levels of education, socio-economic backwardness and stagnant or low level of population growth.[2] The Baiga are one of the oldest aboriginal tribes of central India and are one of the primitive tribes in Madhya Pradesh. Information on the TB situation in this tribal community is not available. Hence, the present survey was undertaken to estimate the prevalence of pulmonary tuberculosis (PTB) amongst them.

MATERIAL AND METHODS

In 1890, an area (Baigachak) in the Dindori district of Madhya Pradesh was officially notified by the British Administration as the land for Baigas.[3] Due to the undulating terrain, physical barriers like thick forest patches, rivulets and hillocks, the Baiga villages are generally isolated from all other communities.

Of the total 8,400 Baiga population in Baigachak, it was decided to cover a 25% random sample of the population keeping in view the limited resources and difficult terrain. Villages in the area

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were selected randomly in order to cover the sample size of 2,100 with the study carried out in five villages during January to March 2008. A complete census of the villages was done by house-to-house visits to register all the individuals. From all the individuals aged 15 years and above, information on the symptoms suggestive of PTB was elicited by health workers and recorded on an individual card in a pre-coded form. Two sputum samples - one spot and one overnight - were collected and examined for AFB by smear microscopy and culture. A person with bacteriologically positive result by either smear and / or culture was considered a case of PTB. All cases were referred to the concerned health authorities for treatment by the RNTCP. The data was analyzed using SPSS package (13.0 version). The study was approved by the Ethics Committee of RMRCT. Informed written consent was obtained from all the individuals.

RESULTS

A total of 2,359 population was covered under the study. Of the 1,410 individuals eligible for screening, 1,374 (97.4%) individuals were screened for symptoms. Of these, 115 (8.4%) individuals were found eligible for sputum collection and the sputum was collected from all of them (Table). Thus, the coverage of above 95% was achieved for both symptom elicitation and sputum collection. The overall prevalence of PTB was found to be 146 (95% C.I: 0 - 318) per 100,000 population.

DISCUSSION

The present study is the first reported community-based TB prevalence study among the Baigas of Baigachak in Dindori district. As no baseline data is available, the findings of the present study form the basis for future work in this area. The prevalence of PTB in the present study was 146 per 100,000 population. TB prevalence surveys carried out in different parts of the country both in the general population[1,4] and in isolated tribal communities[5-8] show wide variation in prevalence rates. A comparable prevalence of 133 was reported amongst the tribal population from Wardha district, Maharashtra.[4] A recently conducted study among tribal population of Madhya Pradesh reported a higher prevalence of 387.[7] Studies in other parts of the country, however, have reported much higher prevalence rates of PTB amongst tribal communities. A very high prevalence of 1,270 has been reported in the Saharia primitive tribal community of Madhya Pradesh, and of 740 in the tribal community of the Car Nicobar of the Andaman & Nicobar islands.[5,6] Reported prevalence in the general population ranges from 144 in Wardha district, Maharashtra State to 1,070 in Raichur district, Karnataka State.[4,8]

Table: Village -wise coverage and sputum positive cases detected amongst Baigas of Baiga Chak

S. No	Village	Total Population Covered	No. eligible for screening	Population screened	No. sputum eligible	No. sputum collected	No. sputum Positives
1.	Khamera	331	186	180 (96.7)	19	19	0
2.	Khaparipani	321	188	182 (96.8)	14	14	0
3.	Dhurkuta	636	345	336 (97.4)	39	39	1
4.	Shital pani	362	226	216 (95.6)	17	17	1
5.	Jaldha	709	465	460 (98.9)	26	26	0
	Total	**2359**	**1410**	**1374 (97.4)**	**115**	**115 (100)**	**2**

* Figures in the parentheses indicate percentage

The results of the present study suggest that PTB is not a major health problem amongst this primitive tribal community at the present time. However, the situation may change if appropriate TB control measures are not taken. At present, this population generally lives in inaccessible forest areas, with poor access to health delivery systems. With the developmental activities in the area, they are now in a phase of transition with major changes in their life-style occurring. If this change does not go hand-in-hand with improved health care delivery, diseases such as TB could increase due to various factors such as increased migration to other areas, etc. The Revised National Tuberculosis Control Programme (RNTCP) has been in operation in the district from 2003–04. With effective implementation of RNTCP over a number of years, a significant decrease in the prevalence of TB disease has been demonstrated in Thiruvallur district, south India.[9] But the performance of RNTCP in Dindori district, as seen from the 2008 annual data is not impressive with a case detection and success rate of 41% and 79% respectively amongst the new smear positive cases.[10] **Effective and strengthened implementation of quality services under RNTCP need to be ensured on a sustained and long term basis in the area.**

The limitations of the study, however, need to be considered while interpreting the results. These are - small population of the tribal group and incredibly small number of cases detected in the survey. Despite this, the study provides important information on tuberculosis in this primitive ethnic group of central India.

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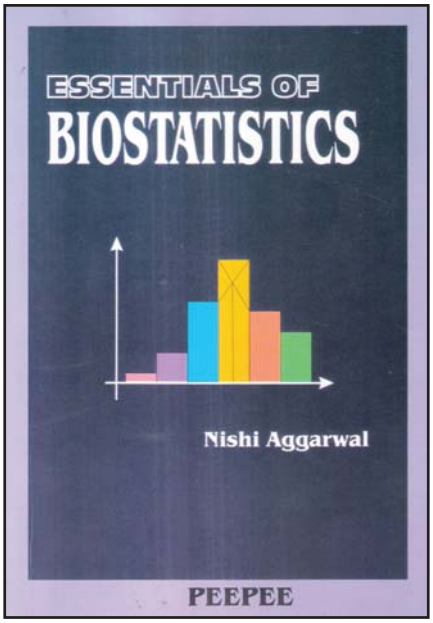
REFERENCES

1. Indian Council of Medical Research. Tuberculosis in India - a sample survey, 1955-58. Special Report Series No. 34. Indian Council of Medical Research, New Delhi, 1959.
2. Tewari DN. Primitive Tribes of Madhya Pradesh – strategy for development. Government of India, Ministry of Home Affairs, Tribal Development Division, New Delhi, 1984.
3. Tiwari SK. Baigas of central India. Anmol publications. New Delhi, 1997.
4. Narang P, Tyagi NK, Mendiratta DK, Jajoo UN, Bharambhe MS, Nayar S. Prevalence of sputum-positive pulmonary tuberculosis in tribal and non-tribal populations of the Ashti and Karanja tahsils in Wardha district, Maharashtra State, India. *Int J Tuberc Lung Dis* 1999;**3(6)**: 478–82.
5. Chakma T, Rao V, Pall S, Kaushal LS, Datta M, Tiwary RS. Survey of pulmonary tuberculosis in a primitive tribe of Madhya Pradesh. *Indian J Tuberc* 1996 ;**43** : 85-9.
6. Murhekar MV, Kolappan C, Gopi PG, Chakraborty AK, Sehgal SC. Tuberculosis situation among tribal population of Car Nicobar, India, 15 years after intensive tuberculosis control project and implementation of a national tuberculosis programme. *Bull World Health Org* 2004; **82**: 836-43.
7. Bhat J, Rao VG, Gopi PG, Yadav R, Selvakumar N, Wares DF. Prevalence of Pulmonary tuberculosis amongst the tribal population of Madhya Pradesh, central India. *Int J Epidemiol* 2009 (in press).
8. National Tuberculosis Institute. Tuberculosis in a rural population of South India; A five year epidemiological study. *Bull World Health Org* 1974;**51**: 473-88.
9. Subramani R, Radhakrishna S, Frieden TR, *et al*. Rapid decline in prevalence of pulmonary tuberculosis after DOTS implementation in a rural area of South India. *Int J Tuberc Lung Dis* 2008;**12(8)**: 916-20.
10. Central TB Division (CTD). Revised National Tuberculosis Control Programme –Annual Status Report. Directorate General of Health Services, Government of India, New Delhi, 2009.

BOOK REVIEW

Essentials of Biostatistics; 2010 (Editor) Dr. Nishi Aggarwal; published by Pee Pee Publishers and Distributors (P) Ltd., Darayaganj, New Delhi.



One of the most important aspects of scientific research in medical practice is careful initial planning analysis of results of any study. Interpretation of results of any study or project requires understanding of the basic principles of Biostatistics and their applications to assess the results and present the same as required for the study or project in a more comprehensive as well as conclusive manner. The medical, both undergraduate as well as post-graduate students, usually find themselves at a disadvantage when planning a protocol and their research study, ascertain about the sample size, designing a project , applying suitable testing for the estimates and interpreting the results as per the study requirements.

The book covers mainly the details of the basic statistics and the various techniques used for the research purposes. Conceptually simple and easy to understand, this introductory textbook is designed to provide the beginners with an insight into the basics of statistics with a working knowledge of this subject- what it is; how and when to apply statistical techniques to decision – making situations and how to interpret the results obtained.

The text includes virtually all the important statistical aids with special emphasis on the basic statistics, basic research methodology, statistical inference and application of the statistical tools for the analysis.

This book written by Dr. Nishi Aggarwal, Statistician, New Delhi Tuberculosis Centre fills up the gap for the medical students and researchers in the field of sciences and will be an asset for them in understanding statistics and its application for the data management and its analysis for their research work.

M.M. SINGH
EDITOR, IJT

ABSTRACTS

Validity of symptoms and radiographic features in predicting positive acid-fast bacilli smears in adolescents with tuberculosis

K.S. Wong, Y.C. Huang, S.H. Lai *et al*. *Int J Tuberc Lung Dis* 2010; **14(2)**: 155-9.

A cohort of 78 adolescents was selected for evaluation with culture or histologically proven pulmonary tuberculosis (PTB) from a tertiary paediatric facility in northern Taiwan. The objective was to assess the validity of clinical features and radiographic findings for predicting positive smears of acid-fast bacilli (AFB) in adolescents with PTB. It was a retrospective descriptive study of adolescents with a confirmed diagnosis of PTB. Clinical symptoms and chest radiographs were assessed. Univariate analysis identified risk factors suggestive of a positive AFB smear, and the adjusted odds ratio (aOR) for these features was calculated using logistic regression. Patients who were AFB smear-positive and those who were smear-negative differed significantly on univariate analysis ($P < 0.05$) with respect to chronic cough, haemoptysis, multilobar or superior segment of lower lobe involvement, cavitations or presence of pleural effusions. Logistic regression analysis revealed that risk factors of positive smear in adolescents with PTB were chronic cough >4 weeks (aOR 13.8, 95% CI 2.3- 83.1), lower lobe involvement (aOR 12.6, 95% CI 1.2- 134.8) and pulmonary cavitations (aOR 7.7, 95% CI 1.0-57.7). For adolescents with PTB, those suffering from chronic cough for >4 weeks, with involvement of the superior segment of the lower lobe or with cavitary lesions, have a greater likelihood of transmitting tuberculosis due to smear positivity.

Outcomes and safety of concomitant nevirapine and Rifampicin treatment under programme conditions.

M. Moses, R. Zachariah, K. Tayler-Smith *et al*. *Int J Tuberc Lung Dis* 2010; **14(2)**: 197-202.

The objectives were to report on 1) clinical, immunological and virological outcomes and 2) safety among human immunodeficiency virus (HIV) infected patients with tuberculosis (TB) who received concurrent nevirapine (NVP) and rifampicin (RMP) based treatment. It was a retrospective cohort study for analysis of programme data from June-December 2007. Of a total of 156 HIV-infected TB patients who started NVP-based antiretroviral treatment, 136 (87%) completed TB treatment successfully, 16 (10%) died and 5 (4%) were transferred out. Mean body weight and CD4 gain (adults) were respectively 4.4 kg (95% CI 3.3-5.4) and 140 cells/mm^3 (95% CI 117-162). Seventy-four per cent of patients who completed TB treatment and had a viral load performed (n = 74) had undetectable levels (<50 copies/ml), while 17 (22%) had a viral load of 50-1000 copies/ml. Hepatotoxicity was present in 2 (1.3%) patients at baseline. Two patients developed Grade 2 and one developed Grade 3 alanine transaminase enzyme elevations during TB treatment (incidence rate per 10 years of follow-up 4.2, 95% CI 1.4-13.1). There were no reported deaths linked to hepatotoxicity. In a rural district in Malawi, concomitant NVP and RMP treatment is associated with good TB treatment outcomes and appears safe. Further follow- up of patients would be useful to ascertain the longer-term effects of this concurrent treatment.

Prevalence of Extensively Drug-Resistance Tuberculosis among patients with Multi-Drug Resistant Tuberculosis.

Surendra K. Sharma, Ninoo George, Tamilarasu Kadhiravan, Pradip K. Saba, Hemant K. Mishra and Mahmud Hanif. *Indian J Med Res* 2009; **130**: 392-5.

Extensively drug-resistant tuberculosis (XDR- TB) is a difficult-to-treat form of multidrug-resistant tuberculosis (MDR-TB). High rates of XDR- TB have been reported from India. We sought to ascertain the prevalence of XDR-TB among patients with MDR-TB treated at a tertiary care centre in New Delhi, India. Case records of patients treated for MDR- TB at the All India Institute of Medical Sciences hospital, New Delhi, between 1997 and 2003 were retrospectively reviewed. All patients underwent a pre-treatment drug-susceptibility testing (DST) to first- as well as second-line drugs. XDR-TB was defined as TB caused by bacilli showing resistance to rifampicin and isoniazid in addition to any fluoroquinolone and to at least one of the three following injectable drugs: capreomycin, kanamycin, and amikacin. A total of 211 laboratory-confirmed cases of MDR-TB were reviewed. The mean age of the patients was 33 ± 12 yr. Fifty one (24%) patients were females. All patients were sero-negative for human immunodeficiency virus infection. Five of the 211 MDR-TB patients had XDR-TB. The prevalence of MDR-TB was 2.4 per cent among MDR-TB patients. Our results showed that XDR-TB was rare among patients with MDR-TB treated between 1997 and 2003 at our centre. Unreported selection bias might have been responsible for the high prevalence of XDR-TB reported in previous hospital-based studies from India.

CCR2, MCP-L, SDF-LA & DC-SIGN Gene polymorphisms in HIV-L infected patients with and without tuberculosis

K. Alagarasu, P. Selvaraj, S. Swaminathan, S. Raghavan, G. Narendran and P.R. Narayanan. *Indian J Med Res* 2009; **130**: 444-50.

Variability in the clinical outcome of persons exposed to and infected with HIV-l and tuberculosis (TB) is determined by multiple factors including host genetic variations. The aim of the present study was to find out whether chemokine, chemokine receptor and DC-SIGN gene polymorphisms were associated with susceptibility or resistance to HIV and HIV-TB in south India. CCR2 V64I (G/A), monocyte chemoattractant protein-l (MCP-l) -2518 A/G, stromal cell derived factor-lá (SDF-lá) 3'UTR G/A and DC-SIGN gene polymorphisms were studied by polymerase chain reaction based methods in HIV-1 infected patients without TB (n=151), with pulmonary TB (PTB) (n=81) and extra-pulmonary TB (n=31), 155 PTB patients without HIV and 206 healthy controls. The genotype frequencies of CCR2 V64I, MCP-l -2518 and DC-SIGN polymorphisms did not differ significantly between the study groups. A significantly increased frequency of GG genotype of SDF-lá polymorphism was observed among HIV+PTB+ patients compared to healthy controls (P=0.009, Pc=0.027). Our data suggest that GG genotype of SDF-lá 3'UTR polymorphism may be associated with susceptibility to PTB in HIV-1 infected patients. A better understanding of genetic factors that are associated with TB could help target preventive strategies to those HIV patients likely to develop tuberculosis.

Adding Moxifloxacin is associated with a shorter time to culture conversion in Pulmonary Tuberculosis.

J-Y. Wang, J-T Wang, T-H Tsai *et al*. *Int J Tuberc Lung Dis* 2010; **14(1):** 65-71.

The objective was to investigate whether adding moxifloxacin (MXF) to the standard anti-tuberculosis regimen can shorten the time to sputum culture conversion in pulmonary tuberculosis (PTB). Adults with culture-positive PTB were divided into two treatment groups by their choice: standard regimen alone (HERZ group) and standard regimen plus daily 400 mg MXF in the first two months (MXF group). Sputum samples were collected thrice weekly in the first eight weeks. The propensity score was calculated to estimate the conditional probability of entering the MXF group. Factors influencing time to culture conversion were investigated using Cox proportional hazards regression analysis stratified by propensity score.

Sixty-two patients were enrolled in the MXF group and 88 in the HERZ group; respectively 51 and 72 completed the study. The regimen was modified before culture conversion in respectively 6 (12%) and 12 (16%; P = 0.47) patients, due to adverse effects. The time to culture conversion was shorter in the MXF group HR 2.1, 95%CI 1.4-3.2). The culture conversion rate after six weeks of treatment was respectively 82 % and 61% (P = 0.011, <0.05/4, calculated using the modified Bonferroni method). Adding MXF to the standard anti-tuberculosis regimen in the first two months was associated with a shorter time to culture conversion, a higher six-week culture conversion rate and reduced transmission of tuberculosis.

Acceptability and Outcome of an internet-based Smoking Cessation Programme

T. Fraser, H. McRobbie, C. Bullen *et al*. *Int J Tuberc Lung Dis* 2010; **14(1):** 113-18

The objective was to evaluate a commercial web-based smoking cessation programme (Smokestop™). Smokestop was offered free of charge to 126 staff members of three Auckland DHBs who wanted to stop smoking. Following a 30 minute face-to-face enrolment meeting, participants were able to log on and use the programme. Nicotine replacement therapy (NRT) was available at no cost. All participants who used the programme at least once were followed up at 1,3 and 6 months after first logging on for assessment of smoking status by self-report verified by carbon monoxide (CO) in expired breath. Of 104 participants who logged onto the programme, 12 (12%) achieved 6-month continuous CO validated abstinence. Participant feedback was largely positive: 46% agreed that the programme had assisted them and 74% stated they would recommend it to other smokers. The concomitant use of NRT was seen as an important component. The results suggest that this internet-based smoking cessation programme is an acceptable method to deliver behavioural support to people who want help in stopping smoking, and that it shows promise as a smoking cessation intervention.

Essay Competition For Medical Students-2010

The Tuberculosis Association of India awards every year a cash prize of Rs. 500/- to a final year medical student in India for an original essay on tuberculosis. The subject selected for the year 2010 competition is 'DOTS plus'. The essay should be written in English, typed double spaced, on foolscap size paper and should not exceed 15 pages (approximately 3,000 words, including tables, diagrams, etc.). Four copies of the typescript should be forwarded through the Dean or Principal of a College/University to reach the Secretary-General, Tuberculosis Association of India, 3 Red Cross Road, New Delhi-110 001, before 30th June, 2010 along with a certificate that the author is a final year medical student.

Indian J Tuberc 2010; 57:121-122

OBITUARY OF WALLACE FOX (1920 – 2010)

Wallace Fox, founder Director of the world-renowned Tuberculosis Research Centre in Madras and a doyen in the field of tuberculosis, passed away on 22nd January this year after suffering from Alzheimer's disease for eight long years. He first visited India in 1955 to explore the feasibility of establishing a tuberculosis research centre in this country, and chose Madras as the site because it was a low-profile city far away from public glare and the corridors of power. Not so well known is the fact that he had contracted tuberculosis himself soon after graduation, and received only 'standard treatment with bed-rest and fresh air' for two years in a hospital. Perhaps there is poetic justice in that he made his name and fame from a home versus sanatorium trial (with anti-tuberculosis drugs) that is often referred to as 'Madras classic'. At the time, when India had as many as 2.5 million TB cases and as few as 23,000 sanatorium beds, this study kindled hopes of the possibility of better management of the disease on a national scale. In 1956 when this trial was initiated, the prophets of doom were many, some saying that randomization was an alien concept in a developing country such as India, while others predicted that no patient could be confined to sanatorium for a year or expected to self-administer drugs at home daily for long periods. To persuade sanatorium patients to stay put, Fox visited them every week and discussed with his clinic staff the next morning any domestic problems arising from their hospitalization. To monitor compliance of patients treated at home, he introduced pill counts at periodic intervals and surprise urine tests to check on drug ingestion; also, he arranged for home visits to retrieve defaulters, initially by health visitors/social workers and clinic doctors, and if all failed he himself went! However, he was quick to realize that such heroic measures would be impracticable under routine programme conditions, and that alternative approaches were therefore necessary.

Fox's understanding of the problem of patient compliance was profound, as can be gleaned from his remarkable review in 1961 at the Amsterdam International TB conference. When he noticed that some patients on daily chemotherapy responded well despite minor degrees of irregularity, he wondered if less frequent chemotherapy but fully supervised, might not be equally effective, a concept suggested by studies of serial serum isoniazid concentrations in patients on daily chemotherapy. This was the rationale of a subsequent randomized control trial of fully supervised twice-weekly treatment and self-administered daily treatment, and the successful outcome of this study, both short-term and long-term, was to become the genesis for the present day global DOTS strategy.

His greatest contribution in the field of tuberculosis in India and other developing countries is the randomized control trial of home and sanatorium treatment, which demonstrated that

(a) Treatment at home for a year was as effective as sanatorium treatment, both initially and over a period of 5 years,
(b) Good diet, plenty of rest and airy, well-ventilated accommodation were not essential for a good outcome, if anti-tuberculosis drugs are taken regularly for a year,
(c) Failures of initial chemotherapy can be successfully treated with second-line drugs, irrespective of whether the initial treatment was at home or in sanatorium, and
(d) No additional risk accrued to close family contacts by treating the infectious tuberculosis patient at home.

Equally important is his path-breaking study in the early 1960s that demonstrated that fully supervised intermittent chemotherapy for a year was at least as effective as standard oral chemotherapy for the same duration. Less known, however, is the fact that he also promoted collaborative Operational Research studies for solving practical management problems such as accurate address-taking of patients in Indian conditions and procedures for retrieving defaulters, as both these were major stumbling blocks for TB programme managers when they tried to implement research-based recommendations under real-life situations.

After five years at Madras, Fox returned to London in 1961. He could well have proclaimed then, like Julius Caesar did in 47 B.C. after vanquishing King Pontus in Asia Minor, "Veni, Vidi, Vici" (I came, I saw, I conquered). But he was very modest and stated that while he may have put Madras on the TB map, it was equally true that the Madras experience had led to his evolution as a mature research worker and prepared him for stiffer challenges. Over the next three decades, even in the midst of his infinite commitments in East Africa, Hong Kong, Singapore, Czechoslovakia and the U.K., he kept very close contact with the Madras Centre in all its research studies, notably of short course regimens, through meticulous correspondence and annual consultant visits. Equally significant were his efforts in installing serious research culture amongst national staff in Madras, and the development of a first rate infrastructure that is today regarded globally as the Mecca for tuberculosis research.

How did Fox do all this? The answer is that, apart from his brilliance, he was a charismatic and assertive leader, who practiced what he preached, respected coworkers regardless of their position, and made even the junior-most cog feel he was indispensable for the success of the Project. It was hardly surprising then that he was able to get the best out of every body. He was unwavering in his basic beliefs but always willing to reason and debate. He had an uncanny ability to make people do what he wanted, but never by authority or dogma, only by reasoning. His middle name could well have been Speed for he was impatient and demanding, and a great believer in the maxim that "Hard work never killed anybody". He strongly encouraged independent thinking and uninhibited expression of new ideas, a sacrilege in the bureaucratic set-up of the 1960s in this country. The hallmarks of his character were scientific honesty (not given to look away from inconvenient or unexpected findings), thoroughness in data analysis (held the view that data could be never over-analyzed), straight-forwardness (no beating about the bush or undue concern about ruffling feathers), and unswerving loyalty to his principles and to fellow workers. His diplomatic skills need special mention. It is often said that "No man can please two masters", but Fox managed to please four (WHO, BMRC, ICMR, Madras State Government) as the Centre was a collaborative venture; it is significant that this unique experience was reported subsequently as a unique case study in management.

Although, Fox's life-time obsession was with tuberculosis, he found time for other things as well. He undertook inhaled therapy trials in asthma and QOL studies in lung cancer, was a voracious reader of books on art and culture, a lover of classical music and Indian cuisine, and a great cricket fanatic. Thus, the facets of Fox's contributions are many – pure science, infrastructure development, and propagation of good research culture in India and other developing countries. Aristotle once said "We are what we repeatedly do. Excellence then, is not an act, but a habit". This dictum summarizes the life and achievements of Wallace Fox who demanded excellence from all who worked with him. For his stupendous contributions, the world at large, and India in particular, must be profoundly grateful.

S. RADHAKRISHNA

PADMA BHUSHAN AWARD TO DR. S.P. AGARWAL

Dr. S.P. Agarwal
M.S.(Surg.), M.Ch.(Neuro), FIMSA, FICS, D.Sc. (h.c.)

President
Tuberculosis Association of India
(Former Director-General of Health Services,
Government of India)

Dr. S.P. Agarwal, President, TAI, has been conferred Padma Bhushan award which is one of the country's highest civilian awards and is given for the distinguished service of high order. This award has been announced on the occasion of the 60th Republic Day.

BIHAR STATE CONFERENCE

The Bihar Tuberculosis Association organized its Fourth State Conference at Patna on 31st January, 2010. It was inaugurated by the Chief Minister Shri Nitish Kumar. The Health Minister of Bihar Shri Nand Kishore Yadav was the Chief Guest. About 100 delegates attended the Conference. Dr. V.K. Arora also attended the Conference and delivered a guest lecture.