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

Indian Journal of Tuberculosis

Published quarterly by the Tuberculosis Association of India

Vol. 60 : No. 4 **October 2013**

Subscription

Inland

Annual	Rs.800
Single Copy	Rs.200

Foreign

For SAARC countries	US $ 30
For South East Asian and Eastern countries	US $ 35
For other countries	US $ 40

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

Contents

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

Indian Journal of Tuberculosis

Vol. 60 **New Delhi, October, 2013** **No. 4**

Editorial

VITAMIN D RECEPTOR POLYMORPHISM & ACTIVE TUBERCULOSIS

[*Indian J Tuberc 2013; 60: 199-201*]

Tuberculosis is a significant public health problem, which is acquiring new forms and a greater threat due to immunodeficiency amongst the host population, leading to drug resistance rise in the pathogens. It is estimated that one third of the world's population is infected with *M. tuberculosis*; but only a minority (5-10%) of those infected, develop clinical disease. This percentage changes from country to country and race to race, suggesting that factors other than bacterial infection determine disease development. Apart from environmental and lifestyle risk factors, host genetic susceptibility is also likely to contribute to activate the disease process. Associations with colorectal cancer and hepatitis B are marked among subjects with vitamin D deficiency and strong data is emerging for active tuberculosis in Vitamin D deficiency subjects.[1]

Vitamin D metabolism can lead to activation of macrophages and subsequently restrict the intracellular growth of *Mycobacterium tuberculosis*. Studies have demonstrated that it regulates the concentration of phagosomes of the macrophage.[2] It is therefore a strong candidate gene for human susceptibility to *M. tuberculosis*. It has been suggested that the link between vitamin D receptor (VDR) polymorphisms and disease susceptibility might be modulated by vitamin D status. Vitamin D also modulates monocyte-macrophage activity in the body and plays a role in human innate immunity for the infectious agent *M. tuberculosis.* Recently published meta-analysis has shown that low serum vitamin D levels are associated with a two-fold higher risk of active TB.[3] Vitamin D exerts its actions through VDR, a nuclear hormone receptor. Polymorphisms in the VDR gene, which may influence VDR activity and subsequent downstream vitamin D-mediated effects, were therefore studied as potential candidates of risk markers for various clinical outcomes.[3-4]

Susceptibility and resistance to PTB are a result of complex interaction between host genes and environmental factors (including extrinsic environment and intrinsic host lifestyle factors). Previous studies have identified some well-known environmental factors related to PTB, such as BCG immunisation, history of exposure to PTB, smoking, alcohol consumption, nutritional status, low socio-economic status, sanitation, hygiene and crowding. Moreover, the importance of host genes in disease susceptibility has been demonstrated. Links have been made between tuberculosis (TB) and deficiency of vitamin D (25-hydroxycholecalciferol) following a number of observations.[4,5] Serum concentrations of 25-hydroxycholecalciferol in patients presenting with TB are on an average lower than in healthy matched controls and the prevalence of TB is higher among those with low serum 25-hydroxycholecalciferol concentrations.[4,5]

The active form of Vitamin D i.e. 1,25(OH)2D3 has a immuno-modulatory activity that activates monocytes and suppresses lymphocyte proliferation, immunoglobulin production and cytokine synthesis, thus playing a role in human innate immunity to certain infectious agents. This may be important in the body's defence against TB, in which the attack of macrophages is a key step in pathogenesis. Vitamin D exerts its actions through binding its receptor, VDR.

A polymorphism at the translation initiation start site of any gene results in different versions of the protein, which differ in length by amino acids. These polymorphisms have been identified as potential candidates for genetic susceptibility to TB, and may provide information on whether vitamin D is important in the prevention of TB. The effects of vitamin D are exerted through a VDR and polymorphisms existing in the VDR gene, may influence VDR activity. Twin studies have strongly indicated that inherited genetic factors play an important role in the development of the disease. Two candidate genes, VDR and natural-resistance-associated macrophage protein 1 (NRAMP1), have recently evoked extensive interest. In 2005, Lewis *et al* performed a meta-analysis to assess the association of pulmonary TB with VDR *Fok*I and *Taq*I polymorphisms.[6] In 2006, Yang and Han summarised the association specifically for VDR *Fok*I polymorphism. The potential roles of VDR and NRAMP1 genetic polymorphisms in the development of PTB have been investigated in various racial groups.[7]

Many studies targeted were underpowered to detect even large differences in risk by genotype. Furthermore, the small number of included studies also restricted the stratified analyses to explore the origin of inconsistencies. There are two main possible explanations for the inconsistencies observed in various studies. First, the potential influence of pertinent environmental factors on different populations may play a role in determining susceptibility to TB. Exposure to sunlight and dietary factors, which can influence serum vitamin D concentrations, are the most plausible effect modifiers. This could also help explain the heterogeneity observed in previous studies. Another explanation is the diversity of genetic background in different populations. It has been reported that genotype frequencies of VDR polymorphisms differ between populations and may contribute to inconsistent associations with disease development.

The associations between vitamin D deficiency and TB may be explained by evidence for an immune-regulatory role for this vitamin. Impaired T cell function, including decreased production of the Th1 cytokines interleukin-2 and interferon c, have resulted from deficiencies of protein, zinc and the active metabolite of vitamin D, 1,25-dihydroxyvitamin D3 (calcitriol). *In vitro* studies have shown that monocytes have receptors for calcitriol and vitamin D metabolites can activate the anti-mycobacterial responses of human monocytes and macrophages, enhancing phagocytosis and granuloma formation.[8]

The incidence of tuberculosis is high in Chronic Kidney Disease (CKD) partly as a result of impaired cell-mediated immunity but if low serum vitamin D levels are also predisposed to tuberculosis, the growing population of people with CKD from underlying causes like DM may need early attention to their body vitamin D levels to mitigate the risk of active tuberculosis.[9] There is therefore the possibility that Vitamin D supplementation can impact in prevention of active tuberculosis.

HIV infection weakens the immune system and increases susceptibility to TB, and this has contributed to the severe TB epidemic in recent decades. VDR polymorphisms have been suggested to be related to host susceptibility to HIV acquisition and disease progression. The knowledge of the basic innate immune defence mechanisms against mycobacterial infection provides hope in the development of safe, simple, and cost-effective strategies to prevent and treat tuberculosis.

In future, many well controlled genetic and clinical studies are required to determine whether VDR polymorphisms play a role in recent emergence of extensively drug resistant tuberculosis which have a global impact. Some of these problems could potentially be overcome by adding vitamin to the treatment of tuberculosis. It seems that vitamin D insufficiency is a frequent finding among community-dwelling elderly, irrespective of latitude, and an almost universal finding among elderly. This may be an important factor leading to activation of latent Tuberculosis in aging adults and will remain a clinical and epidemiological challenge. Atypical clinical manifestations of tuberculosis in older persons can result in

delay in diagnosis and initiation of treatment; thus, unfortunately, higher rates of morbidity and mortality from this treatable infection can occur. Thus, the routine fortification of diet with vitamin D for elderly should be addressed in our national programme. HIV status may therefore influence the association observed for VDR polymorphisms and TB, and should be considered with caution in future studies.

There are racial variations in the allelic frequency distribution for the five investigated polymorphism markers and there may be genetic differences between males and females, which means we need more linkage studies and a gene-related locus study to elucidate the contribution of Vitamin D in Tuberculosis. Thus, further studies are required to investigate the possible interaction of specific physiological pathways in the development of tuberculosis. This knowledge will lead to a better understanding of the immunological pathways in tuberculosis, and offer new insights into tuberculosis treatment and prophylaxis. The interaction between environmental factors , host genetic factors, Vitamin D and other modifying factors leading to active tuberculosis should be addressed in future studies.

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Original Article

TUBERCULOSIS IN HIV CO-INFECTED PATIENTS- A STUDY AT TERTIARY CARE HOSPITAL, AMRITSAR (INDIA)

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(Received on 6.9.2012; Accepted after revision on 29.7.2013)

Summary

Background: The dual epidemic of tuberculosis and HIV is a significant problem in the developed and developing countries. Tuberculosis is the most common human immunodeficiency virus related opportunistic infection in India and caring for patients with both diseases is a major public health challenge.

Aim: The aim of the present study was to record the different clinical patterns of tuberculosis in HIV co-infected patients as a function of CD4+T cell count.

Material and Methods: The study was a retrospective analysis of the HIV-TB co-infected patients admitted in the Chest and TB Hospital, Government Medical College, Amritsar (Punjab) during the calender year 2011.

Results: Out of total 47 HIV sero-positive patients (n=47), 36 were males (76.59%) and 11 females (23.41%) of age group 14 to 51 years. Cough was the most common presenting symptom (72.34%).A large number of patients were diagnosed as having pulmonary tuberculosis (48.94%). The other diagnoses were tubercular meningitis (n=4), pleural effusion (n=4), tubercular lymphadenopathy (n=2), pneumothorax (n=2), hydropneumothorax (n=2) and abdominal tuberculosis (n=2). A total of 34 (72.34%) patients were having a CD4+T cell count of $\leq$200.

Conclusion: The manifestations of tuberculosis in HIV infected patients are quite varied and generally show a different pattern as a function of CD4+ T cell count. Co-infection with HIV infection leads to difficulties in both diagnosis and treatment of tuberculosis. High degree of suspicion of tuberculosis, with astute clinical and laboratory evaluation is the key for early diagnosis and management. **[*Indian J Tuberc 2013; 60:* 202-207]**

Key words: Tuberculosis, HIV, Manifestations, Co-infection, CD4+T cell.

INTRODUCTION

Tuberculosis, known to mankind since ages, is an infectious bacterial disease caused by *Mycobacterium tuberculosis* that spreads almost exclusively by the respiratory route, primarily involving the lungs. HIV infection, on the other hand, acquainted to man in the last decades of the last century only, is a viral disease, spreads by various routes and is notorious for causing immune suppression in the body.The dual epidemic of tuberculosis and HIV is a significant problem in the developed and developing countries. The HIV pandemic has altered both the epidemiology of tuberculosis and the measures for approaches to its control. WHO estimates that more than 7 million people, 98% of whom are in the developing world, are co-infected with HIV and tuberculosis[1].

The incidence of tuberculosis in HIV infected patients is about a hundred-fold than that in the general population[2].It is estimated that 60-70% of HIV-positive persons will develop tuberculosis in their lifetime[3].Approximately, 50% of adult Indian population is infected with *Mycobacterium tuberculosis* and the spread of HIV infection has led to a potentially explosive increase in the number of cases of tuberculosis[3]. About 1.8 million new cases of tuberculosis are occurring annually in India, whereas the pool of HIV-infected individuals is quite large[4]. Tuberculosis is the only major opportunistic infection in HIV infected individuals which can

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spread through the air from a HIV positive person to a HIV negative person[1].

India is accounting for one-fifth of the world's new tuberculosis cases and the estimated prevalence of HIV in the adult population is 0.36%. Presently, about 5% of new tuberculosis cases in India occur in people with HIV co-infection. An HIV sero-positive person infected with tuberculosis is 30 times more likely to develop TB disease than someone who is TB infected but is HIV seronegative[1]. The report, 'Together we will end AIDS', released for the 19th International AIDS Conference in Washington shows that 34.2 million people were living with HIV in 2011. Of them, India housed 2.4 million, the largest infected population after South Africa[5].

Tuberculosis is well known for its variable presentations and progression in different persons or even in the same person at different occasions, which remains a source of confusion for clinicians. Presence of co-infection with HIV, another devastating disease, further blurs the scenario and thus making the diagnosis still difficult.What is most unfortunate is the fact that tuberculosis goes unrecognized and inadequately treated in as many as two thirds of all HIV positive people with tuberculosis. HIV associated tuberculosis is more difficult to diagnose due to several reasons including frequently negative sputum smears, atypical radiographic findings, higher prevalence of EPTB especially at inaccessible sites and resemblance to other opportunistic pulmonary infections and so on.

AIM

Keeping in view the enormity and seriousness of the prevailing situation, the aim of the present study was to elucidate the different manifestations of tuberculosis in HIV co-infected patients as a function of CD4+T cell count.

MATERIAL AND METHODS

The study was a retrospective analysis of the HIV-TB co-infected patients admitted in the Chest and TB Hospital, Amritsar (Punjab) during the year 2011 (from 1st Jan to 31st Dec 2011). The medical records of these patients were extracted and analysed in terms of socio-economic status, history of illness, history of addiction, other related investigations including CD4 counts and radiological abnormalities. HIV infection was diagnosed using Rapid kit tests (SD Bioline HIV ½ 3.0 Rapid kit for screening and confirmed using COMBAIDS-RS Advantage-ST HIV 1+2 Immunodot Test Kit and HIV 1/2/0 Tri-line Human Immunodeficiency Virus Rapid Test Device). CD4+T cell counts were determined by flow-cytometry technique using BD FACSCount™reagent kit. ART was started for eligible patients and was guided by baseline and 6-monthly CD4+T cell counts in accordance with the National ART guidelines[6].

Following investigations were done to establish the diagnosis of tuberculosis:

a). Ziehl-Neelsen (ZN) staining of sputum for acid-fast bacilli (AFB) from given sample was performed as per RNTCP recommendations at the designated microscopy centre (DMC) located within the hospital.

b). Histopathological demonstration of typical caseous granulomatous reaction.

c). Suggestive clinical profile including cough and/ or haemoptysis, fever, night sweats, weight loss or the added features suggestive of TB concerning the involved site.

d). The diagnosis of extra-pulmonary tuberculosis was based on the added features suggestive of TB concerning the involved site with supportive evidence in the form of pleural/ ascitic fluid analysis showing lymphocytic exudative effusion and CSF showing lymphocytic pleocytosis with hypoglycorrhachia (low CSF glucose).

Revised National Tuberculosis Control Programme (RNTCP) recommends sputum microscopy as a tool for diagnosing pulmonary tuberculosis. Although sputum culture for

Mycobacterium tuberculosis is the gold standard diagnostic tool, it requires specially trained staff and facilities, also is time-consuming with a heavy financial burden and is not recommended in the RNTCP guidelines.

RESULTS

The total number of patients with a known HIV reactive status were 47 (n=47) with 36 males (76.59%) and 11 females (23.41%) patients of age group ranging from 14 to 51 years. Most of the patients [n=34; (72.34%)] were from rural areas. The patients' symptoms at presentation are shown in Table1. The most common symptoms at presentation were cough (72.34%), fever (68.09%), breathlessness (68.09%), loss of appetite (63.83%) and weight loss (59.57%). A minor number of patients presented with symptoms like pain chest, loose stool, neurological manifestations like altered sensorium, vomiting, etc. A history of addictions among male patients revealed drug abuse (61.11%), of whom 22.22% were intravenous drug abusers, alcoholism (50.0%), smoking (19.44%) and tobacco chewing (27.78%). None of the female patients gave history of any kind of addiction.

A large number of patients were diagnosed as having pulmonary tuberculosis (48.94%) on the basis of sputum smear microscopy and radiological examination. The other diagnoses were tubercular meningitis (n=4), pleural effusion (n=4), tubercular lymphadenopathy (n=2), pneumothorax (n=2), hydropneumothorax (n=2) and abdominal tuberculosis (n=2). Seven (14.9%) patients came out to be non-tubercular and responded to symptomatic treatment. One patient each was found also to be suffering from diabetes mellitus and chronic renal failure.

One HIV positive patient while on Anti Tubercular Treatment (ATT) and having a CD4+T cell count of < 200 and on Anti Retroviral Therapy (ART) since four weeks presented with increase in the lymph node size, pyrexia and was diagnosed as a case of Immune Reconstitution Inflammatory Syndrome (IRIS). The patient responded to Non-Steroidal Anti Inflammatory Drugs (NSAID) without stopping ATT. Two patients while on ATT presented with icterus and deranged liver functions. ATT had to be stopped for two weeks and patients treated symptomatically until the liver function test (LFT) became normal. Eight patients were on the previously treated regimen as per the RNTCP guidelines for reactivation of pulmonary tuberculosis. Four patients died while in hospital.

The most common radiological finding on chest X-ray was observed to be diffuse pulmonary infiltrates (53.19%); the others were fibrosis (25.53%), cavity (17.02%), pneumothorax and hydropneumothorax (n=4), pleural effusion (n=4), miliary tuberculosis (n=2),

Table 1: Symptoms at presentation

Symptoms	Number of patients (n=47)
Cough	34 (72.34%)
Fever	32 (68.09%)
Breathlessness	32 (68.09%)
Loss of appetite	30 (63.83%)
Pain chest	08
Loose stools	08
Neurological manifestations	05
Vomittings	05
Abdominal pain	02
Swelling in neck	02
Yellowness of eyes	02
Weight loss	28

Table 2: Radiological features

Chest X-ray findings	Number of patients
Pulmonary infiltrates	25 (53.19%)
Fibrosis	12 (25.53%)
Pleural effusion	04
Cavity	08
Miliary tuberculosis	02
Pneumothorax	02
Hydropneumothorax	02
Consolidation	02
Normal chest X-ray	02

Table 3: Distribution of pulmonary and extrapulmonary tuberculosis

CD4+ count	PTB	TBM	PLEF	Abdominal TB	Disseminaed TB	TB LAP	Pneumo/ Hydropneumothorax
≤200	19	04	03	1	2	2	03
>200	04	0	01	1	0	0	01

N.B: A)The revised National AIDS Control Organization guidelines recommend the baseline CD4+T cell count of <350 for initiation of ART.

B) PTB: Pulmonary tuberculosis; TBM: Tubercular meningitis; PLEF: Pleural effusion; TB LAP: Tubercular lymphadenopathy.

and consolidation (n=2) as shown in Table 2. Two patients were having a normal chest X-ray but were found to be sputum smear positive. Three patients who were initially sputum negative turned out to be sputum smear positive by sputum induction method. A total of 34 (72.34%) patients were having a CD4+T cell count of ≤ 200 (Table 3).

DISCUSSION

The HIV and tuberculosis co-infection has come to be known as a deadly duet, a difficult and frequently fatal combination[7]. Tuberculosis is the most common opportunistic infection in HIV infected persons in several countries, including India[7]. Twenty-five to 65% of HIV infected persons have been reported to have active tuberculosis of one organ or the other in developing countries[4].

Asymptomatic, subclinical tuberculosis, with negative findings on a sputum smear and chest radiography and positive culture results, is a common feature of HIV-associated tuberculosis and may account for 10% of cases in regions in which tuberculosis is endemic.[8-10] Up to 25% of patients presenting for HIV care in such regions have undiagnosed active tuberculosis.[11] Therefore, screening for tuberculosis is recommended for all patients with HIV infection to identify patients with active disease. The presence of any one of four symptoms (cough, fever, night sweats, or weight loss) has been shown to have sensitivity in the range of 80% for identifying patients in whom further diagnostic evaluation is warranted, even in resource-constrained regions.[12]

Unlike other opportunistic infections, tuberculosis can occur at any stage of HIV disease, and its manifestations depend largely on the degree of immunosuppression. When the CD4+T cell count was >200 cells/cumm, the disease was more likely to be upper lobe open cavitatary/infiltrative disease; as immunosuppression increased, atypical pulmonary and extrapulmonary (especially meningeal, disseminated, lymphadenopathy) tuberculosis became progressively more common. Maniar *et al* reported that infiltration was seen among 62.5%, hilar lymphadenopathy in 17.5%, pleural effusion in 16.5%, and consolidation in 7.5%. Pericardial effusion was seen among 3.0% and miliary shadows in 1.5%.[13] In another study, Padyana M *et al* reported that infiltration (39%) followed by consolidation (30%), cavity (11%), and lymphadenopathy (9%) was seen with CD4 less than 200 and infiltration (37.5%) followed by cavity (25%) and miliary (25%) with CD4 above 200.[14] The radiographic findings in our study were consistent with these studies.Constitutional symptoms of fever and night sweats were usually present. The clinical presentation depended mainly on immune function. 4.26% patients with smear positive pulmonary tuberculosis had normal chest X-rays. Confirmation of the clinical diagnosis when the immune system was relatively preserved, was by sputum microscopy. However some patients have to be subjected to the sputum induction for a successful smear microscopy examination, emphasizing the significance of this cost-effective technique in diagnosis of tuberculosis.

A new molecular diagnostic test called Xpert MTB/RIF assay detects *M. tuberculosis* complex within two hours, with an assay sensitivity that is much higher than that of smear microscopy.[15] In HIV infected patients, the test has a rate of case detection that is increased by 45%, as compared with smear microscopy.[16] At present this technique is being used in the diagnosis of drug resistant tuberculosis as per the RNTCP's guidelines on programmatic management of drug resistant tuberculosis in India.

Tuberculosis leads to an increase in HIV replication and accelerates progression of HIV infection, with attendant high mortality. Early initiation of ART results in a reduction in mortality; among patients with tuberculosis who do not receive ART, those with very low numbers of CD4+ cells have a high short-term risk of death.[17-19] WHO recommends that ART be started within the first eight weeks after the initiation of tuberculosis treatment and that patients with a CD4+ cell count of less than 50 per cubic millimeter receive ART within the first two weeks.[20]

Fortunately, response to ATT in HIV positive patients was good. However, treatment of tuberculosis, at times was complicated by drug interactions and overlapping toxicities associated with ART and ATT when therapy for both infections was concomitantly administered. The most common adverse effects encountered in this study were gastrointestinal disturbances and drug-induced hepatotoxicity. Literature depicts that ATT induced hepatotoxicity occurs fourfold higher in HIV-TB co-infected patients than in seronegative patients[1].

The study findings revealed the wide prevalence of drug abuse of all kinds (oral, intravenous and sniffing) including that of cannabis and opioids with opium, 'bhuki', morphine, crack or cocaine, etc. amongst male patients, rather to gigantic proportions despite being illegal. It is probably because of the long international porous border abutting this region, with drug trafficking incidents. Tobacco smoking is less prevalent probably because of the prevailing religious constraints.

CONCLUSION

The manifestations of tuberculosis in HIV infected patients are quite varied and generally show a different pattern as a function of CD4+ T cell count. Co-infection with HIV infection leads to difficulties in both diagnosis and treatment of tuberculosis. High degree of suspicion of tuberculosis, with astute clinical and laboratory evaluation is the key for early diagnosis and management.

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Original Article

SPINAL TUBERCULOSIS: A STUDY OF THE DISEASE PATTERN, DIAGNOSIS AND OUTCOME OF MEDICAL MANAGEMENT IN SRI LANKA

BMGD Yasaratne[1], SNR Wijesinghe[2] and RMD Madegedara[3]

(Received on 19.9.2012; Accepted after revision on 9.5.2013)

Summary

Background: Sri Lanka has an intermediate burden of tuberculous disease. Most patients with spinal tuberculosis (STB) are managed with medical treatment alone as advanced surgical facilities are not freely available.

Objective: To describe the clinico-demographic and imaging pattern of STB and to assess the outcome of medical treatment in the local setting.

Design: Descriptive case series.

Methods: All patients diagnosed with definite or probable STB, had their symptomatology and investigations recorded. They were followed up with anti-TB treatment (ATT) according to standard guidelines. An initial six-week tapering course of steroid was given when there was an evidence of neural involvement.

Results: Of 32 patients with STB, backache was the commonest presenting feature (92%). Nine had lower limb neurological deficits. Uni-focal upper lumbar involvement was the commonest disease pattern noted in the series. High ESR (84%) and Mantoux positivity (53%) were frequent. 72% had end-plate changes on imaging. 53% had paraspinal soft tissue components. The triad of backache, high ESR and end-plate and/or paraspinal disease on CT/MRI showed a diagnostic sensitivity of 81.2%. Response to ATT was satisfactory in 87%. Poor neurological response was seen among some with large paraspinal collections or extensive vertebral damage at diagnosis.

Conclusion: This study showed that backache over one month, high ESR and specific CT/MRI features helped diagnosis of STB, in the absence of definitive evidence. Medical management alone, comprising a prolonged course of ATT with an initial steroid cover when indicated, appeared to be safe and effective in the local setting for uncomplicated STB. **[*Indian J Tuberc 2013; 60:* 208-216]**

Key words: Tuberculous spondylitis, Pott's disease, Outcome

INTRODUCTION

Tuberculosis (TB) is one of the oldest diseases affecting mankind and has been found in skeletal remains from the ancient mummies of Egypt and Peru.[1] The disease is caused by the bacillus *Mycobacterium tuberculosis*, and occasionally by *Mycobacterium bovis*, and *Mycobacterium africanum*. It is the most common infectious disease causing deaths in humans. TB is presently a global epidemic with over two billion people, equal to one-third of the world's population currently estimated to be infected, with 8.8 million new TB cases identified worldwide and 1.4 million deaths annually.[2]

Pathogenesis of skeletal TB is related to reactivation of haematogenous foci or spread from adjacent paravertebral lymph nodes. Weight bearing joints (spine 40%, hips 13%, and knee 10%) are most commonly affected.[3] Spinal TB (STB, Pott's disease) is uncommon in developed countries, but is encountered frequently in the endemic regions. This often involves two or more adjacent vertebral bodies and destruction of these causes spinal deformities and neurological complications.[4]

Sri Lanka falls in the World Health Organization (WHO) category of intermediate burden countries where, despite an effective national programme for TB control and mass scale immunoprophylaxis with BCG vaccination, TB still remains a growing public health issue with over 9000 new cases being detected annually.[5] Despite a high rate of suspicion, diagnostic confirmation of STB is challenging in most instances due to the

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indolent nature of the illness and difficulty in obtaining tissue samples. Therefore clinical picture and imaging play an important role in the diagnosis.

OBJECTIVES

The primary objective of the study was to describe the clinico-demographic and imaging pattern of STB in a series of patients in the study setting. The secondary objective was to assess the treatment outcome of the disease within the limited resources.

DESIGN AND SETTING

Descriptive case series from Respiratory Unit II, Teaching Hospital, Kandy (2006-2010).

METHODOLOGY

We recruited all patients diagnosed with definite or probable STB from September 2006 to March 2010 (n=32) at Respiratory disease clinic, Teaching Hospital, Kandy.

Diagnosis of STB

The diagnosis of STB was made based on a combination of clinico-radiological and biochemical factors. The criteria for diagnosis (modified from Ching-Yun Weng, *et al* [6]) were as follows; (1) Symptoms over one month duration; (2) specific features on MR/CT imaging; (3) exclusion of alternative spinal disease; (4) raised inflammatory markers or positive Mantoux testing or both. If patients had only the above criteria, they were categorized as probable STB; if also showed confirmatory microscopical or histopathological evidence on examination of paraspinal aspirates or tissue biopsy when performed, they were categorized as definite STB (Table 1). Both probable and definite STB categories were included in this study, while others with possible STB, but did not fulfil the above diagnostic criteria were excluded.

To exclude alternative spinal disease to the maximum possible extent, we performed blood cultures, myeloma screening and malignancy screening in all and Brucella serology, bone biopsy, cerebrospinal fluid examination and isotope bone scanning when indicated. The response to a four-week trial of Anti-TB Treatment (ATT) was also considered as retrospective supportive evidence.

Data acquisition

We reviewed the clinic records and spinal images of all subjects. Patient symptomatology, demographic details, co-morbidities, past TB status, contact status and examination findings including weight, neurological complications and gibbus deformity at the time of diagnosis were recorded. We also documented the investigation results including inflammatory markers, Mantoux reading, sputum status and imaging details. We reassessed all patients at a special follow-up clinic.

Treatment and follow up

We treated diagnosed STB patients with a prolonged regimen of ATT according to the WHO

Table 1: Criteria used in the diagnosis of spinal tuberculosis (modified from Ching-Yun Weng, *et al* [6])

Diagnostic criteria for spinal tuberculosis
1. Symptoms exceeding one-month duration
2. Specific imaging features on MRI/CT spine
3. Exclusion of alternative spinal disease
4. Raised ESR / Mantoux positivity (or both)
5. Paraspinal aspirates showing acid-fast bacilli
6. Histology of tissue biopsy demonstrating granulomatous inflammation or caseation
Definite STB
Fulfil all criteria 1-4 and 5 or 6
Probable STB
Fulfil criteria 1-4 only
Possible STB
Fulfil criteria 1-3 only

and national guidelines.[7,5] Treatment regimen comprised isoniazid, rifampicin, pyrazinamide and ethambutol in a two-month intensive phase and isoniazid and rifampicin for a further ten-month continuation phase. If the initial CT/MR imaging showed neural involvement, they were commenced on oral dexamethasone (equivalent to prednisolone 0.75–1 mg/kg/d) for three weeks which was tapered off over the next three weeks. All patients with neurological complications and extensive disease on imaging were put on spinal corsets (external bracing) and advised on initial immobilization, after taking neurosurgical and/or orthopaedic opinion as appropriate.

We closely followed up all the patients in the tuberculosis clinic with monthly reviews. They were assessed in relation to disease complications such as formation of gibbus deformity, development of new neurological symptoms or signs and pathological fractures. We also monitored them for possible treatment complications with regular clinical examination for early liver disease, periodic visual assessment and frequent monitoring of blood counts and liver biochemistry (transaminases and bilirubin levels).

The response to treatment was monitored with symptomatology, weight, inflammatory markers and serial spinal x-rays. Due to lack of resources, we were unable to perform post-treatment MR/CT imaging in all to assess radiological resolution. However, repeat MRI were performed in seven patients, including all with residual neurological clinical weakness. Nine others including all with extensive pre-treatment bony destruction underwent repeat CT at the end of ATT. At the time of analysis, all patients had completed the one year regimen of ATT with a 17.6 month average post-treatment follow up.

Ethics/confidentiality

Since the patients were recruited retrospectively, ethical approval was not required. However we took informed patient consent at follow-up clinic. All records were kept confidentially.

RESULTS

32 patients (19 males) with average age of 48 (range 08-76) years were diagnosed with definite (n=3) and probable (n=29) STB over three and half years. Another patient who had vertebral body and pedicle destruction with positive Mantoux was empirically commenced on ATT, but was subsequently diagnosed to have spinal metastases on isotope scanning and bone biopsy.

One had coexisting smear negative pulmonary TB. Two others had identifiable contacts. None of the patients had confirmed past TB or previous anti-tuberculosis treatment. Diabetes mellitus seen in seven patients was the commonest co-morbidity. All were negative on HIV screening.

Imaging findings of STB

All patients underwent initial spinal X-ray imaging. End plate changes, disc narrowing and paraspinal masses were the main abnormalities revealed in the majority (Table 2). Three (9%) had apparently normal plain spinal x-rays.

Table 2: Common X-ray abnormalities noted in the cohort of patients with spinal tuberculosis

Specific X-ray features	No. of patients (n=32)
End plate sclerosis / erosion	17
Disc space narrowing	12
Paraspinal soft tissue shadows	10
Spinal angulation / vertebral collapse	7
Lytic areas in vertebral bodies	2
Apparently normal x-rays	3

(Note: Many had more than single x-ray abnormality)

Seventeen patients had undergone diagnostic MRI of the spine and eleven had CT of the spine, while both MRI and CT scans were performed in four, depending on the availability of facilities and patient affordability at the time of diagnosis. Only two patients had more than one distant spinal regions involved simultaneously (multi-focal disease). Of the remaining thirty with uni-focal disease, twenty one (70%) had two adjacent vertebral segments involved, while a single segment was involved in six (20%) and over three adjacent segments were involved in three (10%).

Lumbar first and second segments were the commonest affected (22%). The other commonly affected regions were lumbar fourth and fifth, thoracic eighth, lumbar third and lower thoracic (ninth to twelfth) segments (Figure 1).

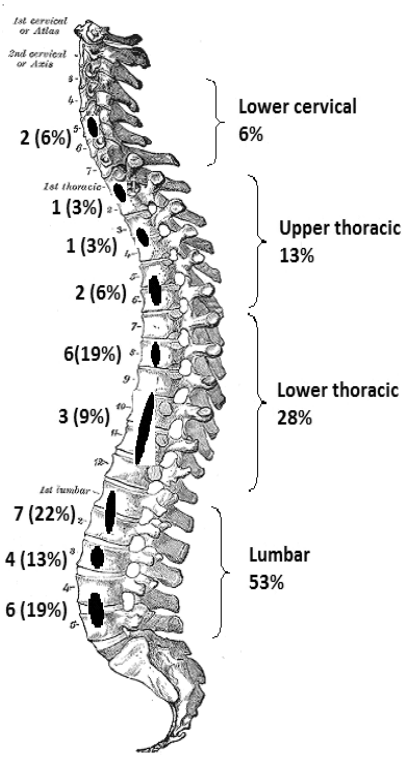


Figure 1: Frequency of involvement of different spinal regions in the cohort with spinal tuberculosis

End-plate sclerosis with or without mild erosive changes was the chief feature noted in 23 (72%) on CT/MR imaging. Three with end plate involvement had erosion of the adjacent vertebral body. Paraspinal lesions were present in sixteen of them, while disc involvement was noted in thirteen. Psoas abscess was seen in seven of them (Table 3).

Six others had extensive involvement of the vertebral bodies and discs. Clinically evident kyphoscoliosis was seen in four of them. Of the remaining three patients, two had isolated lytic areas of the vertebrae and one had paraspinal lesions alone. Out of all patients, ten had imaging features suggestive of spinal cord or root involvement.

The earliest feature of spondylitis was end plate involvement and oedema, which was detected as low intensity over the disc on T1 and high intensity on T2 weighted MR images (Figure 2A). CT also

Table 3: Specific MRI and CT imaging findings observed in the group at diagnosis

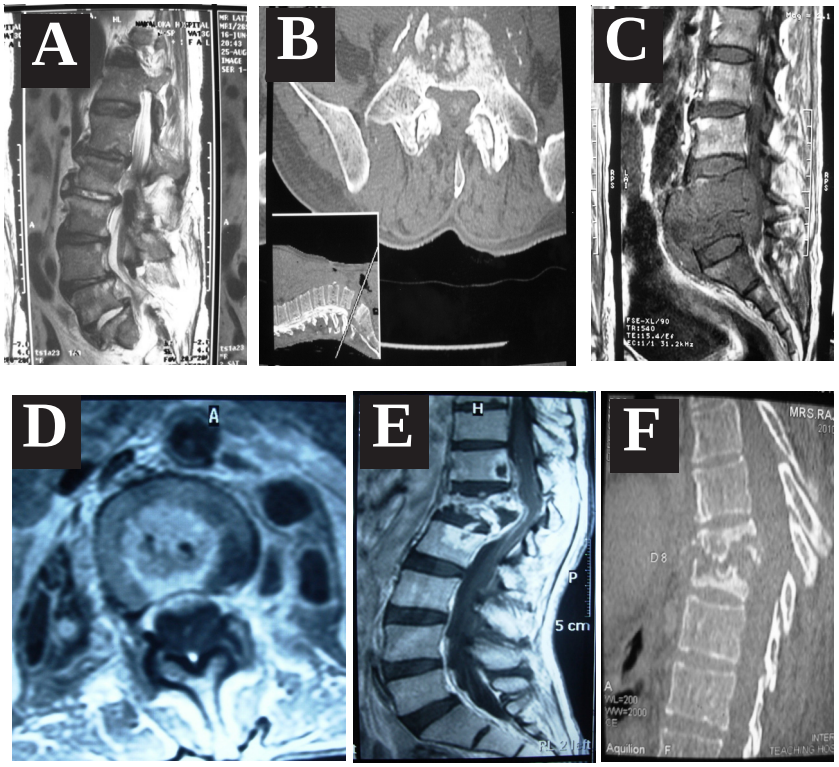
Specific MRI / CT abnormality	MRI (n=17)	CT (n=11)	MRI & CT (n=4)	Total (n=32)
End-plate sclerosis/erosion	10	9	4	23
Paraspinal soft tissue masses	11	4	2	17
Unilateral psoas abscess	3	1	2	7
Bilateral psoas abscesses	1	-	-	
Discitis	9	-	4	13
Extradural cord compression	1	2	-	10
Intrathecal root compression	1	-	1	
Spinal cord & root compression	3	-	2	
Body destruction / Vertebral collapse	1	3	2	6
Isolated lytic areas in vertebral bodies	1	1	-	2

(Note: Many had multiple radiological abnormalities)

showed end plate sclerosis / destruction at a later stage (Figure 2B). Subligamentous spread of infection (Figure 2C) with paraspinous abscess formation, reduction of disc height with discitis, extension to psoas muscle (Figure 2D) or epidural space and neural compression (Figure 2E) were the other specific imaging features looked for, which were better seen on contrast MRI. Large paraspinous or vertebral body abscesses, vertebral body destruction (Figure 2F) and disc space narrowing were also seen on CT images. Plain x-ray images were helpful in identifying fusiform paraspinal soft tissue swelling and vertebral collapse in advanced cases.

Clinical features and diagnosis

Backache was the commonest presenting feature in 29 (91%) patients, while fever, loss of appetite and weight, night sweats, kyphoscoliosis, lower limb neurological deficits and sphincter disturbance were also noted (Figure 3). Of the six patients with extensive vertebral and disc involvement on imaging, four had clinically evident kypho-scoliosis, with two of them having gibbus deformity. Even though evidence of spinal cord or nerve root compression was seen in ten of the MRIs, only five of them had clinical neurological weakness. Four



***(A)**T_2 weighted sagittal MR images showing high signals in Lumbar 2nd & 3rd vertebral bodies. **(B)**CT axial imaging of lumbar spine showing end plate and vertebral body destruction. **(C)**T_1 weighted sagittal MRI shows subligamentous spread of infection. **(D)**Axial MR image shows bilateral psoas abscesses. **(E)**Sagittal MRI showing lumbar 1st destruction, thoracic 12th intra-osseous abscess formation, epidural extension and cord compression. **(F)**CT shows gross vertebral destruction with gibbus deformity.*

Figure 2: Specific imaging features of spinal tuberculosis

others had neurological deficits without MRI showing neural compression.

Average ESR at presentation was 82mm/hour, with 15 (47%) having ESR over 100mm/hour. ESR was over twice the age related expected maximum [i.e age÷2 for males and (age+10)÷2 for females] in 27 (84%). Mantoux test was positive (induration >10 mm) in 17 (53%).

In this group, seven underwent aspiration of paraspinal collections and two patients had

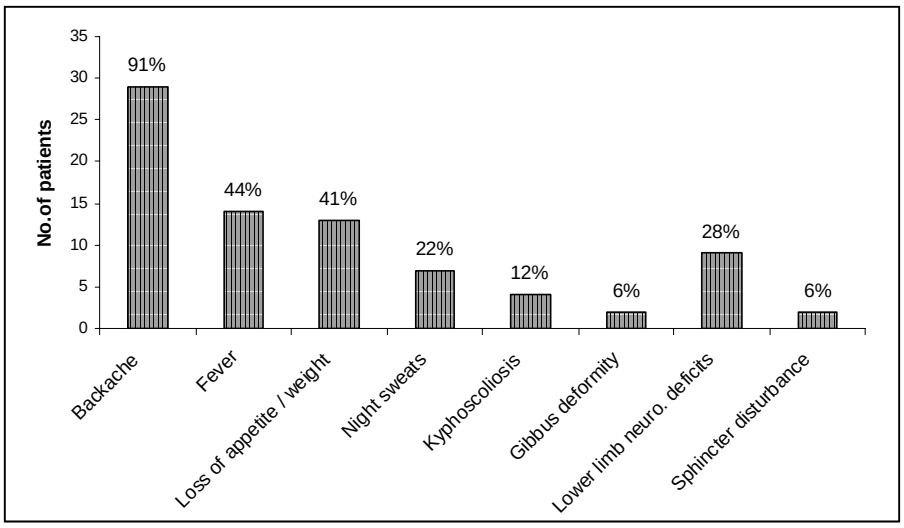


Figure 3: Commonest clinical features observed in spinal tuberculosis

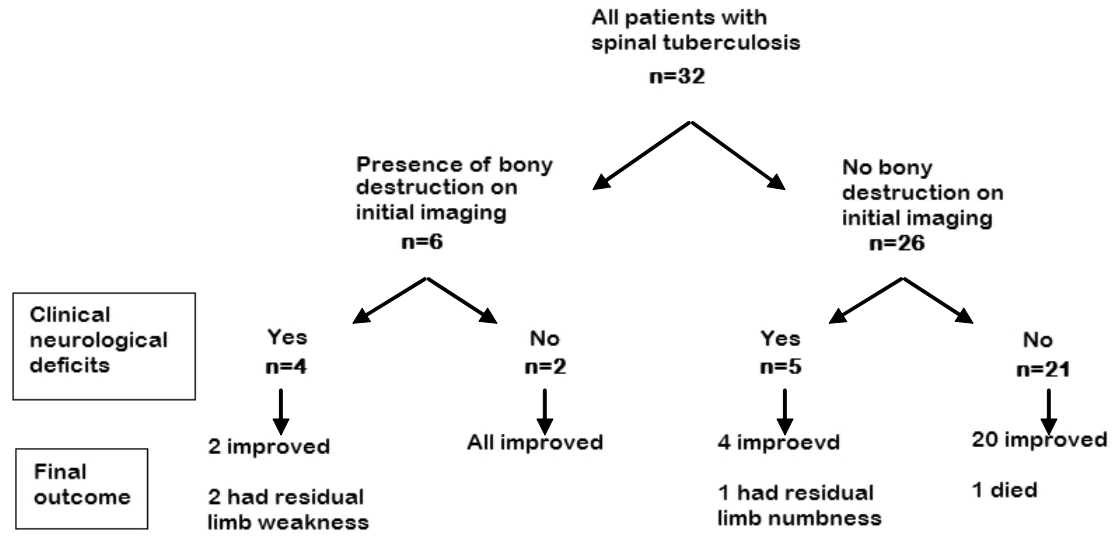


Figure 4: Final outcome with medical management in spinal tuberculosis

visible mycobacteria in paraspinal aspirates. Three underwent CT guided vertebral biopsy, of whom one had histopathological evidence of tuberculosis, while the others were inconclusive. In the diagnostic work up of STB, the triad of chronic backache for over three months, high ESR of over twice the expected maximum and end plate involvement or paraspinal lesions on CT/ MR imaging was present in 26, giving a sensitivity of 81.2%.

Treatment Outcome

One patient, a 62-year-old male with co-existing diabetes, died at home during the second month of the treatment course, where the exact cause of death was not elucidated. His records did not reveal evidence of adverse effects to medication. Remaining 31 completed one year of ATT. Average post-treatment follow up was 17.6 months (median 16; range 36; inter-quartile range eight months) at the time of analysis.

Drug induced hepatitis was noted in two, requiring transient withdrawal of treatment, but no other major ATT related adverse effects were noted in the cohort.

Of the 31 followed up, 28 patients (90%) had symptomatic improvement with weight gain over 2 kilograms in 24 (75%). Of the 27 patients with high pre-treatment ESR, normalization during treatment was seen in 23 (85%).

Of the nine patients with neurological limb deficits, recovery at the end of treatment was full in six, while three had residual lower limb root pain (Figure 4). Two of them had extensive spinal destruction with vertebral collapse on initial MRI, which persisted at post-treatment MRI. The other had persisting paraspinal abscesses, in spite of ATT full course. Four other patients who had initial bony destruction had clinical improvement with medical treatment and external bracing and showed radiological resolution with some residual changes on repeat CT.

DISCUSSION

We observed that STB commonly affects males of late middle age. It is of interest to note that only one patient had possible pulmonary TB and only two others had possible TB contacts in this series. This is in contrast to the findings by Nussbaum, *et al* in their 29-patient series in United States, where they have noted past TB in 52%, concurrent PTB in 10% and had identified family contacts in 17%.[8] This may be a reflection of high rate of sub-clinical infection in an endemic setting and also past BCG / exposure immunity.

Diagnosis of STB is challenging worldwide, due to lack of advanced radiological and operative facilities in the developing world and due to low suspicion in the developed world.[9] Imaging features of STB have been well described.[10,11] Certain features have been identified as sensitive for STB rather than pyogenic osteomyelits, which include calcified, large paravertebral abscesses, multi-focal disease, subligamentous spread, relative sparing of the disc and heterogenous MRI intensity.[12] Even though these features help diagnosis, at the earliest stage, there will only be oedema or infective changes at the cartilage end plate, seen only on contrast MRI. Fungal spondylitis, though uncommon, shares many radiological features with STB, including skip lesions, paravertebral lesions and disk sparing, and causes diagnostic confusion.[13]

Even though multi-focal disease or skip lesions are known to be more specific for STB, this is seen less commonly. We have noted only two such patients and similarly CY Weng, *et al* had seen only one in their series of 38 patients.[6] Lumbar and thoracic spine were the commonest regions involved universally[4,14], which is noted in this series as well. However, we noted that lumbar involvement was slightly commoner in aged patients, while thoracic involvement was more in the young. Involvement of two adjacent vertebrae is commonly seen (70% in our series and 68% by Weng, *et al* [6]). However Weng, *et al* also observed 10% having four or more congruous vertebral disease, which was never a finding in our series.

Intramedullary tuberculoma is a rare entity (2:100,000 TB cases) noted in some case series[8,15], but we did not encounter any. However, since eleven of our patients had diagnostic CT scanning alone, there is an initial chance of missing such lesions and therefore follow up MRI or post-myelogram CT would be required in suspicious cases.

Relative disc sparing is considered virtually pathognomonic for STB. However, mild reduction of disc height can be seen early in the disease, as was seen in about 40% of patients in this series. This apparent disc narrowing is postulated to be due to herniation of the disc in to partially destroyed vertebral bodies, rather than true spread of infection.[10]

Chronic backache has been the commonest symptom in STB collectively (79-100%)[4,13,16], including in this series. Even though ESR is commonly elevated, 16% in the series had normal ESR for age. We had a lower rate (28%) of neurological deficits, compared to 76% observed in the US series by Nussbaum, *et al*.[8]

We performed pre-treatment MRI scans in all patients with neurological deficits, but found evidence of neural compression only in five out of nine patients. We noted that some patients with subtle neurological findings, such as isolated regional sensory impairment or isolated reflex impairment, may not show such MRI changes. Therefore in this series, initial MRI did not well correlate with subtle neurological involvement.

Seven out of nine patients with clinical neurological weakness at diagnosis, had post-treatment MRI. Of them, three with residual weakness had either skeletal collapse or large paraspinous abscesses. Repeat MRI scans were normal in the rest.

Several surgical approaches for complicated STB have been discussed in the literature, but further prospective studies are required to evaluate surgical outcome.[17] In our series, only two patients with neurological deficits had significant paraparesis, while others had varying involvement to a lesser degree. There are many practical reasons, some unique to a limited resource setting, such as delayed presentation, lack of facilities to arrive at a microbiological / histological diagnosis, heavy neurosurgical workload, patient non-consent and cost that hindered prompt surgical management in advanced cases. External bracing and medical management were offered to all and majority had good clinical and biochemical response to treatment. None underwent initial internal fixation and non-responders to adequate medical therapy were referred back for definitive surgery.

CONCLUSION

The triad of backache over one month, high age adjusted ESR and end plate/paraspinal disease on CT/MR imaging was useful to diagnose STB with a sensitivity of 81.2%, in the absence of definitive microbiological or histological evidence in majority, in the local setting. Uni-focal involvement of upper lumbar region was the commonest disease pattern. CT visualized the disco-vertebral lesions and the paravertebral abscesses, while MR imaging was useful to determine the spread of disease to the soft tissues and the spinal canal. However, initial MRI at diagnosis did not well correlate with subtle neurological involvement.

Diagnosis based on clinico-radiological and biochemical factors in the absence of definitive evidence, appears to be safe and effective in the limited resource local setting with an intermediate burden of tuberculous diseases. A prolonged course of ATT together with a four-to-six-week steroid cover when neurological involvement is present, appear to be safe and effective for STB without advanced skeletal destruction or extensive paraspinous spread at presentation. Nevertheless, these patients with probable disease, especially the ones with less typical imaging findings, should be closely followed up to exclude an alternative diagnostic possibility. Aspiration of large paraspinous abscesses should be encouraged, as this will aid in the microbiological confirmation of diagnosis and may have therapeutic benefits.

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Original Article

GENDER DIFFERENCES IN HEALTH CARE SEEKING BEHAVIOUR OF TUBERCULOSIS PATIENTS IN CHANDIGARH

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(Received on 17.10.2012; Accepted after revision on 11.6.2013)

Summary

Background: Gender is a social determinant of health. In view of the substantial burden of tuberculosis (TB), it is important to look into the gender issues related to utilization of services.

Aims: To find out gender differences in health care seeking behaviour of tuberculosis patients.

Methods: A cross sectional study, using integrated mixed method, was conducted in Chandigarh (India). Systematic random sample was used to interview 109 TB patients (54 men and 55 women) from eight randomly selected health institutions.

Results: More women (40%) resorted to home remedies or medicines without prescription at the onset of symptoms compared to men (13%). More men (87%) consulted qualified medical practitioners compared to the women (60%). Consultations from private doctors were more common among men. Mean delay in diagnosis was more in men (60 days) than women (33 days). Main reasons for delay, in men and women respectively, were late referral by doctor (37% *vs* 26%), long distance to health institution (29% *vs* 28%), prolonged use of self-medication (30% *vs* 26%), and financial constraints (7% *vs* 17%). More women (20.8%) reported missing a prescribed dose of treatment as compared to men (11.1%). However, 10% men were on re-treatment compared to none of the women.

Conclusions: Delay in diagnosis was more in men than women. More delay occurred due to delayed referral by doctors among men and due to financial constraints among women. Hence, gender differences in health care seeking behaviour should be kept in mind while selecting programme strategies. **[*Indian J Tuberc 2013; 60:* 217-222]**

Key words: Adherence, Delay, Gender, Health Seeking Behaviour, Health Service Utilization, Tuberculosis

INTRODUCTION

Tuberculosis (TB) accounts for about 2.5% of global burden of disease[1] and 26% of preventable deaths[2]. Each year, 8.74 million people develop tuberculosis and nearly two million die of TB. India accounts for one-third of the global TB burden with 1.8 million developing the disease each year and nearly 0.4 million dying of TB annually[3].

Global estimates indicate that women account for about 3.6 million cases of TB. The situation is more complicated in countries like India where TB kills more women than any other infectious disease and more than all causes of maternal mortality combined. Moreover, about 100,000 women are rejected by their families each year because of TB, strongly impacting their children and families[4]. In India alone, 30,000 children leave school annually, on account of their parents' TB[5].

In view of the substantial burden of TB and specific health needs of women, it is important to look into the gender issues related to the utilization of health services under the Revised National Tuberculosis Control Programme (RNTCP). This study was conducted to find out gender difference in health care seeking behaviour of TB patients.

MATERIAL AND METHODS

The cross sectional study was conducted in Chandigarh Union Territory among TB patients selected from the clinics. In 2009, Chandigarh had

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two TB Units and 48 Peripheral Health Institutions (PHIs) where 2264 TB patients were registered (1366 men and 898 women).

Sample size was calculated taking into account estimated adherence to treatment of 80% among TB patients with a 10% absolute precision and design effect of 1.8. Multistage stratified systematic random sampling method was used for selection of 120 study participants (60 men and 60 women). Out of 48 PHIs, eight institutions were selected randomly. From the selected PHI, every 4th or 5th client was selected for interview, as during the duration of one interview three to four clients would consult the doctor and leave the clinic. Out of the 25-30 clients visiting the clinic on a single day, 8-10 clients were interviewed. Thus data were collected from 54 men and 55 women. Six men and five women did not consent for the interview. All ethical principals were followed.

Integrated mixed method approach was adopted to collect qualitative and quantitative data using a pre-tested interview schedule. The first part of interview schedule was open-ended for narratives and second part had semi-structured questions designed to seek information on demographic profile and socio-economic factors, patterns of health care seeking behaviour, access to health care services and treatment adherence.

Quantitative data (socio-demographic, delay in diagnosis, and adherence to treatment) was analyzed using SPSS 16 for Windows (SPSS Inc. Chicago, IL). The qualitative data were analysed to trace behaviour pattern from the onset of symptoms till approaching the clinic and reasons for delay in seeking care, if any. Statistical test of significance (chi square for categorical data and t test for quantitative data) were used to find differences in health care utilization among men and women.

RESULTS

The mean age of respondents was 32 years. Men were younger than women. More than 80% of respondents were married. More men than women were living in urban area. Most of the men and women were working in the unorganised sector as street vendors, construction workers and household helpers/ maids (Table).

At the onset of symptoms, more women (40%) than men (13%) resorted to home remedies or medicines without prescription. More men (87%) directly consulted qualified medical practitioner as compared to women (60%) (Figure). The proportion of consultation with private doctors was higher among men than women. Multiple consultations before starting the DOTS were higher among men than women (on an average 1.4 and 1.1 respectively). There was a mean delay of 48 days from the onset of symptoms to diagnosis through sputum test. However, once sputum test was found positive, the DOTS treatment was started immediately. The mean delay was 60 days in men and 33 days in women. Though men are considered to be independent, resourceful and mobile, but had more delay in diagnosis than women.

Table: Demographic and Socio-Economic Profile of Respondents

Characteristics	**Males N=54**		**Females N=55**	
	No.	**%**	**No.**	**%**
Age (in Years)				
15-30	28	51.9	33	60.0
30-45	16	29.6	19	34.6
45-60	10	18.5	3	5.4
Area of Residence				
Urban	30	55.6	22	40.0
Rural	14	25.9	21	38.2
Slums	10	18.5	12	21.8
Marital Status				
Married	46	85.2	50	90.9
Unmarried	8	14.8	5	9.1
Literacy				
Illiterate	13	24.1	12	21.8
Up to level 10	36	66.7	39	70.9
Graduate and above	5	9.2	4	7.3

When asked to specify the main reasons for delay in the diagnosis of TB, 37% men and 26% women responded that the doctor from whom they were taking treatment did not refer them timely, 29% men and 28% women mentioned the long distance from home to health institution as the reason; 30% men and 26% women waited for relief in symptoms by the use of home remedies/over the counter medications, and 7% men and 17% women reported financial constraints.

Narratives provided insight into the reasons for delay in seeking care. Among women, main reason for the delay was the 'customary practice' of using home remedies or medication without prescription. A 25-year-old woman elaborated "*I got fever; for two months I took home remedies and medicines from chemist shop but there was no improvement, so I went to a government hospital where after testing they told me that I have TB and sent me to a TB clinic near my place of residence"*. Another 18-year-old woman mentioned *"I had fever for about two months with cough, took medicines from chemist shop, but didn't get relief, so came to dispensary to take medicines, they sent me to government hospital, where my sputum was tested and X-ray was done and I was told that I am having TB*".

It is not only customary that women take home remedies or indulge in taking medicines without prescription but there are underlying social norms that push them to do so. As expressed by 30-year-old woman, "*I started having fever about one year ago; I took medicines without consulting the doctor as my parents-in-law did not take me to a doctor. I came to my parents' house who took me to the hospital. I was admitted for 15 days, was tested and learnt that I had TB"*. Another 19-year-old female said *"I lost appetite and had vomiting with fever, went to private doctor, didn't get relief then I went to government hospital, then my father took me to a bigger hospital, they tested my sputum, blood and did X Ray etc. and told me that I have TB*". It shows that access to resources as well as the right to take decision for seeking treatment even in case of a married adult woman, is bound by social structure. Statements like "Parents-in-law never took me to doctor" or "father took me for treatment" by women respondents are indicative of 'restricted mobility' of women.

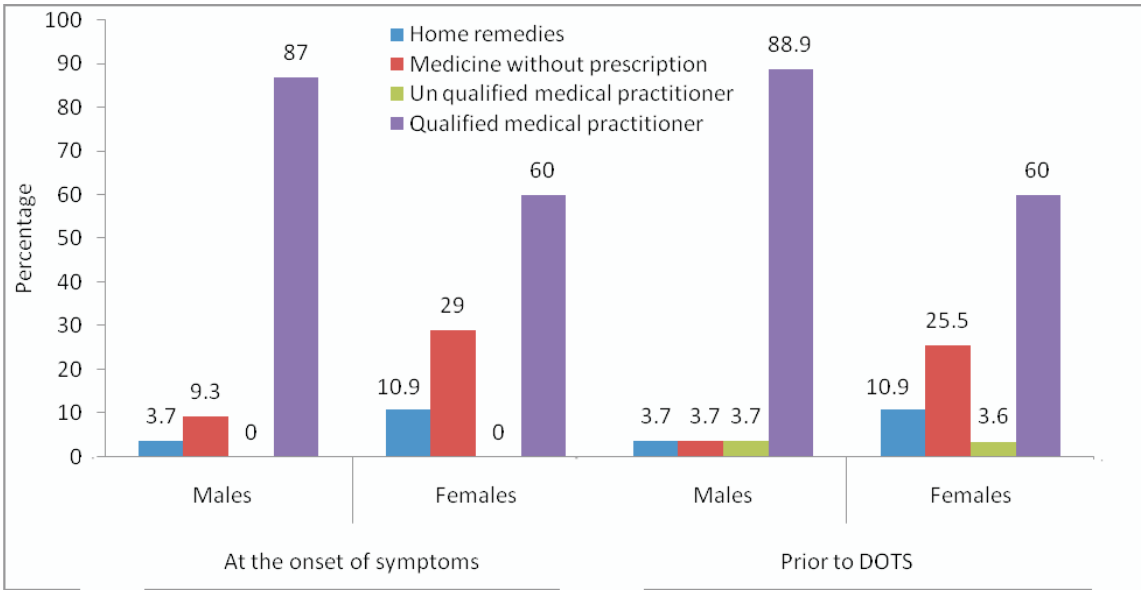

Figure: Source of Health Care in TB Patients

On the other hand, most of the men often did not share their health problems at home till the symptoms worsened leading to delay in diagnosis. A 33-year-old male respondent stated "*I was a habitual alcohol drinker, I got fever, cold and cough for which I took medicines from the chemist store but did not bother to tell this to my family; there was no improvement so I went to a government hospital and got sputum tested, where I was found to be positive for TB*". Another 25-year-old man mentioned "*I got fever so took medicines from a pharmacist for about one and a half month but cough persisted and deteriorated….. my family came to know about my problem when blood started coming on coughing, they panicked and I went to a government hospital where I was diagnosed for TB…. referred to TB centre*".

As far as treatment is concerned, more women (20.8%) reported missing a prescribed dose of treatment as compared to the men (11.1%). Among women, 87.3% reported that they would stop treatment at the advice of doctor; 9.1% could not say when they would stop treatment; and 3.6% would stop when the symptoms would disappear. In contrast, all men stated that they would stop treatment only at the advice of the doctor.

While all women were on anti-tuberculosis treatment for the first time, 9.5% men were on treatment for the second time as they had left the treatment for reasons like migration. A man in his late 20s narrated "*10 years ago when I was in my home town I had blood in sputum with cough and was on TB treatment for three months after which I left the treatment as family migrated to different town..... six months ago I started having cough with blood again so got sputum test done from a government hospital, referred to TB centre, near my residence*".

DISCUSSION

Gender encompasses characteristics of men and women that are distinct from those that are biologically determined[6]. Present study revealed gender differentials in care seeking behaviour and reasons thereof using integrative mixed method design[7] while other studies had studied gender differentials using quantitative method only[4,8]. The treatment seeking behaviour was different among men and women, although their demographic and socio-economic profile was similar.

More women than men had started with home remedies at the onset of symptoms. On the other hand, most men started the treatment from the qualified private service providers that has implications on timely diagnosis. Similar results have been found in another study as well[9]. Women autonomy has always been an issue of concern which has been reflected in other studies too[4,8]. Women in our study were dependent on the family members for seeking care. More women than men had cited financial constraints. Women had no alternative but to resort to home remedies or medicines from a nearby shop without prescription. Under-reporting of TB in women has been attributed to barriers women face in accessing TB care by some[10-12], whereas others ascribe it to the natural epidemiology of the disease[13,14].

It was recognised that DOTS is a better health intervention over self-administered regimen as it led to better monitoring and follow-up of cases[15,21]. However, despite the existence of DOTS centres within 1-3 kilometres in Chandigarh, distance was reported as one of the reasons for delay in seeking treatments in both men and women. It reflects that *emic* and *etic* perceptions for the 'distance' need to be considered while planning and implementing programmes[16]. Of course, the services should be acceptable and affordable[15] but at the same time it is essential to determine the 'extent' to which observed gender differences in TB rates arise from distinctive 'obstacles' faced by men and women[3]. Poverty, one of the socio-economic factors being a major reason for not seeking proper treatment both by men and women is not a new finding[17], however, decision to go for treatment outside home had been a limiting factor for women compared to men, similar trends have been seen in other studies as well[15,18,19]. Domestic social responsibilities have been reported to hinder women's access to the limited resources[20]. However, present study found that 'limited power to take decisions' and 'restricted mobility' are the major reasons for not accessing care among women.

More delay in diagnosis occurred among men than in women in the present study. The reasons for delay also differed in men and women. Men often did not share their illness with the family until symptoms worsened when family members persuaded them to seek care from a government health facility. More men than women had approached private doctors at the onset of symptoms. Delay in referral by the doctor was cited by more men than women as one of the main reasons for delay. It is interesting to note that more men had contacted a doctor at the onset of symptoms than women and delay in diagnosis was also more among them. It seems that private doctors continue the treatment and do not get the sputum test done until symptoms worsen.

Another study using mixed method approach reported differential delay in seeking treatment, however, it reported more delay in women than in men but the reason for delay was reported to be only lack of awareness.[23] The delay in diagnosis and treatment among tuberculosis patients and reasons thereof have been reported in a multi-country study also where the mean delay between the onset of symptoms until treatment ranged from 1.5 to four months in different countries. Socio-demographic, economic, stigma, and time to reach the health facility, seeking care at non specialized individuals, and multiple consultations before diagnosis had been the main factors for delay[22]. Stigma, attached to TB, as shown by other studies[19] seems to have taken a back seat as nobody reported about stigma in our study.

Women under treatment tended to miss prescribed dose of treatment more often than men. But on the other hand, some men abandon the treatment. More men were on re-treatment regimen than women in this study. A multi-centric study showed that more women dropped out during the course of diagnosis, while men diagnosed with TB did not adhere to the treatment[9].

CONCLUSIONS

The delay in diagnosis occurred more often among men than in women. Most women continue to be on home remedies because of their 'restricted mobility' and 'lack of decision making power' while more men continue to avail services from private doctors without 'sharing their illness to the family' until symptoms worsen. More women miss doses of treatment than men while some men abandon the treatment altogether leading to more re-treatments in them compared to women. Hence, gender differences in health care seeking behaviour should be kept in mind while selecting strategies for reducing delay in diagnosis and improving adherence to treatment.

ACKNOWLEDGEMENTS

We acknowledge the contribution of Dr Supreet Kaur, for her contribution in preparation of interview schedule and in initiation of data collection and Dr. P. K. Shridhar, State TB Officer, for his enormous support in conducting this study.

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Original Article

EXPOSURE TO CETYL PYRIDINIUM CHLORIDE AND LOSS OF INTEGRITY OF CELL WALL OF MYCOBACTERIA

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(Received on 21.3.2013; Accepted after revision on 25.6.2013)

Summary

***Background*:** Cetyl pyridinium chloride (CPC) liquefied sputum was shown to reduce AFB smear positivity presumably damaging cell wall of *M. tuberculosis.*

Settings: National Institute for Research in Tuberculosis, Chennai, (Tamil Nadu).

***Objective*:** To assess the cell wall damage of mycobacteria in CPC liquefied sputum, by Transmission Electron Microscopy (TEM) and mycobacteriophage adsorption studies.

***Methods*:** Pooled sputum sample from smear positive pulmonary TB patients was homogenized and liquefied with CPC. It was examined in TEM daily for four days, to assess cell wall damage of *M. tuberculosis*, and photomicrographs were taken. *M. smegmatis* mc^2155, treated with CPC, was infected with mycobacteriophage (phAE129) to study phage adsorption on cell wall and plaque formation. CPC untreated sputum and *M. smegmatis* formed controls.

***Results*:** Photomicrographs showed that cell wall of *M. tuberculosis* was intact in controls and damaged in CPC preserved sputum for 96 hours. Plaque formation was seen and absent respectively in CPC untreated and treated *M. smegmatis* cells.

***Conclusion*:** Exposure to CPC damaged the cell wall of *M. tuberculosis* within 96 hours. Mycobacteriophage failed to form plaques after *M. smegmatis* mc^2155 was treated with CPC implying inhibition of phage adsorption on damaged cell wall and thus providing a clue for poor staining and smear positivity in microscopy. **[*Indian J Tuberc 2013; 60:* 223-226]**

***Key words*:** *M. tuberculosis*, Cetyl pyridinium chloride, Cell wall, Mycobacteriophages, TEM

INTRODUCTION

Smithwick *et al* demonstrated the use of Cetyl Pyridinium Chloride (CPC) for the transportation of sputum samples from remote health centres to tuberculosis reference laboratories for mycobacteriological investigations[1]. WHO recommended the use of CPC for transportation of sputum samples from smear positive patients in drug resistance surveillance (DRS) to mycobacteriology laboratories.[2,3] However, liquefaction of CPC preserved sputum was found to reduce the AFB smear positivity, especially in fluorescence microscopy using auramine phenol (AP) staining.[4,5]

Scanning electron microscopy studies showed that formation of cell wall of mycobacteria was impaired due to interference of mycolic acid synthesis by INH and subsequent loss of acid-fastness of mycobacteria.[6] Phage adsorption studies provide evidence that phages infect their respective bacterial hosts by adsorbing onto specific receptors located on the cell wall of the bacteria.[7,8] When cell wall integrity is damaged due to chemical or physical pressure, phage adsorption is expected to be affected. The knowledge of possible damage of bacterial cell wall by chemicals and impairment of phage adsorption on damaged cell wall prompted us to study the cell wall damage and formation of plaques in CPC exposed *M. tuberculosis* by transmission electron microscope (TEM) and by phage adsorption studies. Reasons for reduction of AFB positivity as it is known that damaged cell wall may be limit the binding of auramine to the cell wall of mycobacteria.[9]

MATERIAL AND METHODS

TRANSMISSION ELECTRON MICROSCOPY

Pooled sputum (~25 ml) from smear positive pulmonary TB patients was homogenized in a mechanical shaker and aliquoted into five parts. The

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first aliquot was processed by modified Petroff's method[10] and formed the control. The second, third, fourth and fifth aliquots were mixed with CPC at a concentration of 7.6 mg/ml (P-CPC 38 mg for 5 ml instead of 75mg in liquid form) and kept at ambient conditions until used. Every day one aliquot was washed twice with distilled water and the deposits were each treated with 125 µl of 20% glutaraldehyde. The deposits were subjected to TEM study with negative staining by Phospho Tungstic acid (PTA) method.[11] In brief, 125 µl of 20% glutaraldehyde was added to the deposit and 2 µl of the deposit was placed on to the copper colloidion coated grid with 400 meshes. The excess was drained off after two hours. A drop of 2% PTA was added and allowed to stand for two minutes. After drying, the grid was examined under TEM (Philips Technai. 10, magnification 24000X). The morphology of bacilli was photomicrographed. The deposit from fifth aliquot was cultured onto Lowenstein Jensen (LJ) medium in addition to TEM. Suspension of *M. tuberculosis* $H_{37}RV$ (equivalent to # 1 McFarland Units) in distilled water was treated with glutaraldehyde and examined under TEM.

PHAGE ADSORPTION ASSAY

Recombinant mycobacteriophage construct, phAE129 was propagated in *M. smegmatis* mc^2155 using Luria–Bertani medium as per protocol and maintained at 4^0 C.[12] Suspension (equivalent to # 4 McFarland Units) of fresh culture (24 hours) of *M. smegmatis* (the experiment is extrapolated to *M. tuberculosis*) was made and aliquoted into two. One aliquot treated with CPC at a concentration of 7.6 mg/ml and the other treated as control were incubated at 37^0C for 24 hours. Lawns of *M smegmatis* treated with CPC and untreated were prepared by mixing 300 µl of the cells with 5 ml of soft agar (0.7%) and poured on Middle Brook 7H9 media (Difco, USA) supplemented with 5% glycerol and 10% albumin dextrose complex (G7H9) plate. Ten fold dilutions of mycobacteriophage were made up to 10^{-5} and about 5 µl from each dilution was spotted on the lawns of *M. smegmatis* mc^2155 treated with CPC and without CPC. The plates were incubated at 37^0C for 24 hours and the formation of plaques was studied.

In a separate experiment, the mycobacteriophage (titre10^9 pfu/ml) was treated with CPC (7.6 mg of CPC/ml) for two hours at 37^0C. Untreated phage was used as the control. About 5 µl of test and control phage were spotted on a lawn of *M. smegmatis* mc^2155 and incubated at 37^0C for 24 hours and the formation of plaques was studied.

RESULTS

TEM EXAMINATION OF *M. TUBERCULOSIS* IN SPUTUM TREATED WITH CPC

The cell wall of *M. tuberculosis* was intact in controls (Fig. 1a). It was increasingly distorted in sputum exposed to CPC for 24 hours, 48 hours and 72 hours (Figs. 1b, 1c and 1d). It was completely degraded after exposure to CPC for 72 hours. The

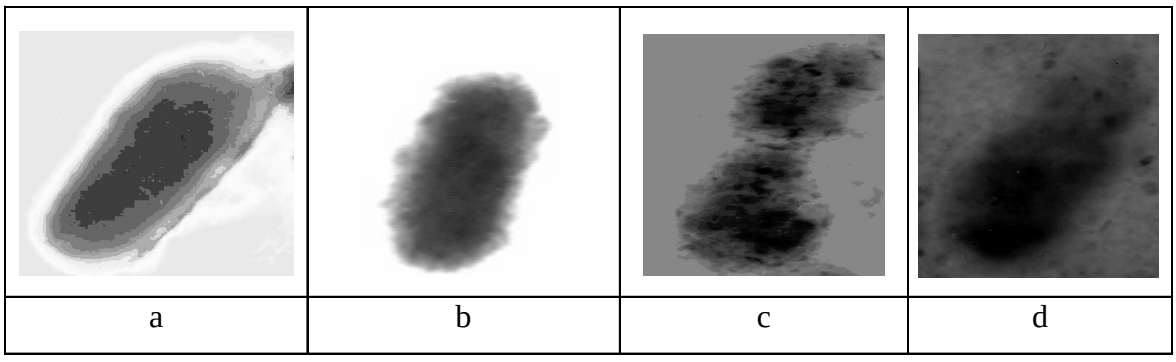

Fig. 1: Transmission Electron Microscopy photomicrographs of *M. tuberculosis* in sputum unexposed to CPC and exposed to CPC (a) Zero Day (b) 24 Hours (c) 48 Hours (d) 72 Hours

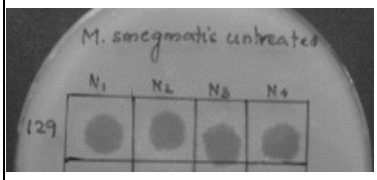	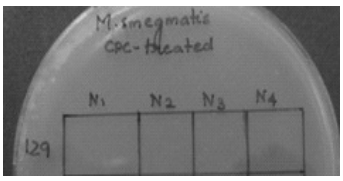	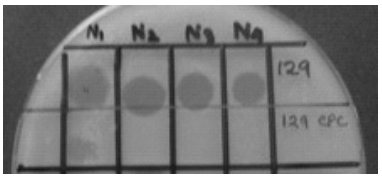
Macro plaques of phAE129 in untreated *M. smegmatis* mc^2155 lawn	No plaque formation in CPC treated *M. smegmatis* mc^2155	No plaque formation with CPC treated phAE129

Fig. 2: Phage adsorption on *M. smegmatis* mc^2155 (a) untreated with CPC (b) Treated with CPC (c) Phages treated with and without CPC

5th aliquot, washed two times with distilled water and cultured on LJ medium, grew *M. tuberculosis* (the morphology of colonies are smooth but no time difference between the growths).

PHAGE ADSORPTION ONTO *M. SMEGMATIS* MC^2155 TREATED WITH CPC

With CPC untreated cells, the lawn formation was thick and distinct macro plaques were formed (Fig. 2a). With CPC treated cells, the lawn was thin and plaques were absent (Fig. 2b). Phage exposed to CPC, did produce normal plaques (Fig. 2c).

DISCUSSION

TEM study revealed that cell wall of *M. tuberculosis* was intact both in sputum sample and in culture suspension not exposed to CPC. It also showed that the cell wall of *M. tuberculosis* was completely damaged in sputum treated with CPC for four days. Nonetheless, the 5th aliquot yielded growth of *M. tuberculosis* on LJ medium. Thus, reduction of AFB positivity in sputum preserved with CPC may be due to damage in the cell wall of *M. tuberculosis*. The damaged cell walls are deterrent to the binding of auramine. However, it is interesting to note that the bacilli with damaged cell wall still resulted in normal growth on LJ medium.

M. tuberculosis possesses unique forms of mycolic acids that are highly sensitive to isoniazid. A close relationship was shown between the synthesis of these specific mycolic acids and the staining ability of the cells.[13] Cells exposed to CPC gradually lose their integrity of cell wall until a complete loss occurred at about 72 hours.

CPC-NaCl preserved sputum yielded less AFB positives in AP method.[5] This could be due to exposure of *M. tuberculosis* to CPC. The reliability of AP method is due to more intensive binding of mycolic acids of the bacilli to auramine and that the bacilli stand out sharply against dark background to allow rapid and accurate screening under low power objective. It can be deduced from the present experiment that bacterial cell wall is damaged after exposure to CPC resulting in the poor staining ability by AP method leading to reduced smear AFB positivity.

Lindberg *et al* demonstrated that phage adsorption was affected due to impaired cell wall synthesis using cell wall deficient forms of Salmonella species.[14] David *et al* showed that addition of colistin inhibited the lytic cycle of the mycobacteriophage D29 in *M. tuberculosis*.[15] In the current study, normal plaque formation was seen in untreated cells demonstrating effective phage adsorption. There was no plaque formation in CPC treated cells. Absence of plaque formation could be attributed to the cell

wall damage in the host due to CPC exposure or inactivation of phages. The latter was tested in the second experiment in which phages were exposed to CPC and made to infect untreated normal host cells.

CONCLUSION

Exposure to CPC results in the damage of *M. tuberculosis* cell wall as observed under transmission electron microscope. Exposure of *M. smegmatis* to CPC impairs phage adsorption. The cell wall damage observed in CPC preserved sputum could be attributable to the poor staining and reduced AFB positivity in microscopy.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Dr. Andrew Chandramohan, Veterinary College, Chennai, for his excellent technical support in Transmission Electron Microscopy studies, staff of Bacteriology Department for their help in laboratory experiments and ICMR-DHR, Government of India, New Delhi for financial support.

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ANNUAL RISK OF TUBERCULOUS INFECTION IN A RURAL POPULATION OF SOUTH INDIA AND ITS RELATIONSHIP WITH PREVALENCE OF SMEAR POSITIVE PULMONARY TUBERCULOSIS

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(Received on 29.4.2013; Accepted after revision on 22.7.2013)

Summary

Background: We conducted a tuberculin survey to estimate the annual risk of tuberculous infection (ARTI) among children in a sub-division of rural Bangalore district. A TB disease survey was conducted in the same area around the same time and has already been published. DOTS strategy is being implemented in the study area since 2002.

Methods: The tuberculin survey was conducted during 2010-2011 among 3838, 5-9-year-old children attending 147 schools selected by simple random sampling. Children were tuberculin tested with 2TU PPD RT23 with Tween 80 and maximum diameter of induration was measured between 48-96 hours. ARTI was computed from prevalence of infection estimated by mirror-image technique. Prevalence of smear positive pulmonary TB estimated during the disease prevalence survey in 2008-10 was used to find out its relationship with ARTI.

Results: Using the observed mode of tuberculin reaction sizes at 19 mm, among surveyed children, prevalence of infection was estimated at 7.3% (CI: 6.5-8.1); ARTI was computed at 1.05%. Considering the mean age of children, estimated ARTI most closely approximated to the year 2008. Every one per cent ARTI was found to correspond to a prevalence of 103 sputum smear positive patients of PTB, which was similar to the ratio of 106 found in the same study area during 1960s.

Conclusion: There has been no change in the relationship between ARTI and prevalence of smear positive pulmonary TB from the pre-DOTS era and thus in the number of children infected by each adult point prevalent case of smear positive pulmonary TB each year suggesting the need for early case detection and treatment. **[*Indian J Tuberc 2013; 60:* 227-232]**

Key words: Tuberculosis, Infection, Risk, Annual, Prevalence, India

INTRODUCTION

Styblo observed that every one percent ARTI corresponded to a prevalence of 80-120 smear positive pulmonary TB (PTB) patients per 100,000 population, based on studies carried out in 13 developing countries including India, during the period 1956-1971 when effective TB control programmes were not in place[1]. The Indian data was obtained from two districts in South India namely Chingleput and rural Bangalore. After introduction of Revised National Tuberculosis Control Programme (RNTCP) adopting Directly Observed Treatment Short course (DOTS) strategy in India during 1990s, the relationship is available only from Thiruvallur district where every one per cent of ARTI corresponded to prevalence of smear positive PTB at 131 (95% Confidence Interval (CI: 118–143)[2]. RNTCP was implemented from 1999 in Thiruvallur district which has been carved out of the erstwhile Chingleput district. In Rural Bangalore where RNTCP was implemented from 2002, we have carried out surveys to estimate prevalence of PTB and ARTI in one of its taluks (taluk corresponds to sub-division of a district). Disease survey during 2008-10 revealed a prevalence of smear positive PTB at 108 (CI: 82-134) among persons above 14 years of age – published data[3]. Results of ARTI survey carried out during 2010-2011 and presented hereunder have been used to derive the relationship between ARTI and prevalence of smear positive patients in Rural Bangalore.

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MATERIAL AND METHODS

Study site and Setting

Nelamangala sub-division of Bangalore rural district is located adjacent to Bangalore metropolitan city.

Study Population

The ARTI survey was carried out among children 5-9 years of age attending class 1 - 4 in the schools located in the taluk. All children irrespective of the presence or absence of BCG scar were eligible for inclusion in the study.

Sampling

Sample size was calculated at 3650 to find out the prevalence of infection within 20% of the true value at 5% level of significance with a design effect of two to account for cluster sampling, considering the expected prevalence at 5.0% (based on results of second zonal level tuberculin survey in southern zone during 2009-10)[4].

Cluster sampling was used for selection of schools. All 336 schools (297 government and 39 private) located in the sub-division were listed serially. Subsequently, schools were selected by using simple random sampling till the number of enrolled children touched 4867 presuming a loss to follow-up of 25% from enrolment to reading due to absenteeism and failure to obtain parental consent. The data on number of children enrolled in each class of individual schools was collected from education department. A total of 147 schools were thus selected.

Field procedures

Fieldwork was carried out by field staff of National Tuberculosis Institute, Bangalore (NTI) having vast experience in tuberculin surveys.

Three visits were undertaken to each school.

On first visit, purpose of survey, nature of tuberculin test and likely adverse effects were explained to school heads. Similar information was provided to parents/guardians by means of printed pamphlets issued to children and their written consent was sought.

On the second visit undertaken 2-3 days later, all eligible children present in the school and whose parents had consented were administered 0.1 ml of 2TU PPDRT23 with Tween 80 (procured from Statens Serum Institute, Copenhagen (SSI) in a single batch) intra-dermally on mid-anterior aspect of left forearm using a disposable tuberculin syringe. Test was recorded as 'satisfactory' if it raised a flat pale wheal with clearly visible pits and well demarcated borders, and 'unsatisfactory' in case of leakage or subcutaneous injection. Date of birth, sex and presence or absence of BCG scar was recorded. Children with fever or recent history of skin rash were excluded from tuberculin testing.

Third visit was undertaken 2 - 4 days later to undertake reading of the reaction between 48-96 hours after tuberculin administration[5]. Reader identified the margins of induration by careful palpation and measured its maximum transverse diameter in millimeters (mm) using a transparent ruler. Reader dictated the reaction size to a secretary for recording. BCG scar status of the child was thus not known to the reader. Most (96%) of the readings were performed by a single reader, in order to avoid inter-reader bias. Parents of children having reaction size of $\geq$10 mm were advised through a letter in local language that in the event of the child having persistent cough or fever for $\geq$2 weeks, recent loss of weight, failure to gain weight or family history of TB in the last two years, the child may be taken to the nearest government health facility to rule out TB.

The study protocol was approved by the Institutional Ethical Committee of NTI.

Statistical methods

Data entered into the computer was verified and analyzed using SPSS version 17.0.

Tuberculin reaction sizes among all children (including those with and without BCG scar, and

doubtful scar), after excluding children with unsatisfactory tests, were plotted to identify the mode of tuberculin reactions representing infection due to infection with tubercle bacilli[6]. Distributions were also plotted by BCG scar status to identify the mode. The anti-mode that separates true tuberculous reactions from cross reactions was ascertained for children without BCG scar[6].

Prevalence (P) of infection was estimated by mirror-image (MI) method in which the proportion of reactions larger than the mode of tuberculous reactions is doubled and added to the proportion at the mode[6]. CI was obtained with binomial exact probability theory

$$CI = P \pm 1.96 \sqrt{\frac{P(1-P)}{n}}$$

[P - estimated prevalence of infection; *n* – Number of children satisfactorily test read]

ARTI was computed from the estimated prevalence of infection by using the following equation [7]:-

$$R_{b+a/2} = 1-(1-P_{b+a})^{1/a}$$

[$R_{b+a/2}$ - ARTI at mid-point between birth of the cohort and mid-point of the survey, b-mean calendar time of the birth of study cohort, a- mean age of children, P_{b+a} - estimated prevalence of infection]

To find out the influence of BCG vaccination if any on ARTI estimates among all children, estimation using MI-method was undertaken by BCG scar status. Estimation among unvaccinated children was also undertaken using anti-mode (AM) method in which all reactions larger than or equal to the anti-mode were labelled as tuberculous [6]. Children tested unsatisfactorily were excluded from analysis.

RESULTS

ARTI Estimates

A total of 4957 children were registered into the survey. Of them, 4066 (82.0%) were tested-3996 satisfactorily and 70 (1.7%) unsatisfactory. Of the satisfactorily tested, reading was undertaken in 3838 (96.1%) children. Overall, 77.4% of the registered children were satisfactorily test read. Of them, 3088 (80.5%) had BCG scar, 743 (20.1%) were without BCG scar and scar was doubtful in 7 (0.1%). Mean age was 7.18 years among all children, 7.17 among children with BCG scar and 7.25 among children without BCG scar.

Frequency distributions of tuberculin reaction sizes among all children presented at figure 1 revealed two modes – one at 2 mm and the other at 19 mm. First mode probably corresponds to cross reactions while the second mode corresponds to reactions due to infection with tubercle bacilli.

Frequency distributions of tuberculin reaction by BCG scar status are presented at figure 2. Both the distributions revealed the mode of reactions due to infection with tubercle bacilli at 19 mm as in the overall study group. Among children without BCG scar, an anti-mode could also be observed at 14 mm.

Using the 19 mm mode, estimated prevalence of infection by mirror-image method, among all children was 7.3% (CI: 6.5-8.1); ARTI was computed at 1.05%.

Prevalence of infection among children without and with BCG scar using the mode at 19 mm was 8.3% (CI: 6.4-10.3) and 7.1% (CI: 6.2-8.0) respectively; the difference was not statistically significant (P value=0.27). ARTI estimates were 1.02% and 1.20% respectively. Prevalence by AM method, among children without scar was 7.1 (CI: 6.3-7.9) with ARTI estimate at 1.02%.

Considering the mean age of children test read, the estimated ARTI would apply to the year 2008.

Relationship between ARTI and Prevalence of smear positive PTB

Considering that the disease survey during 2008-10 carried out in the same area revealed a

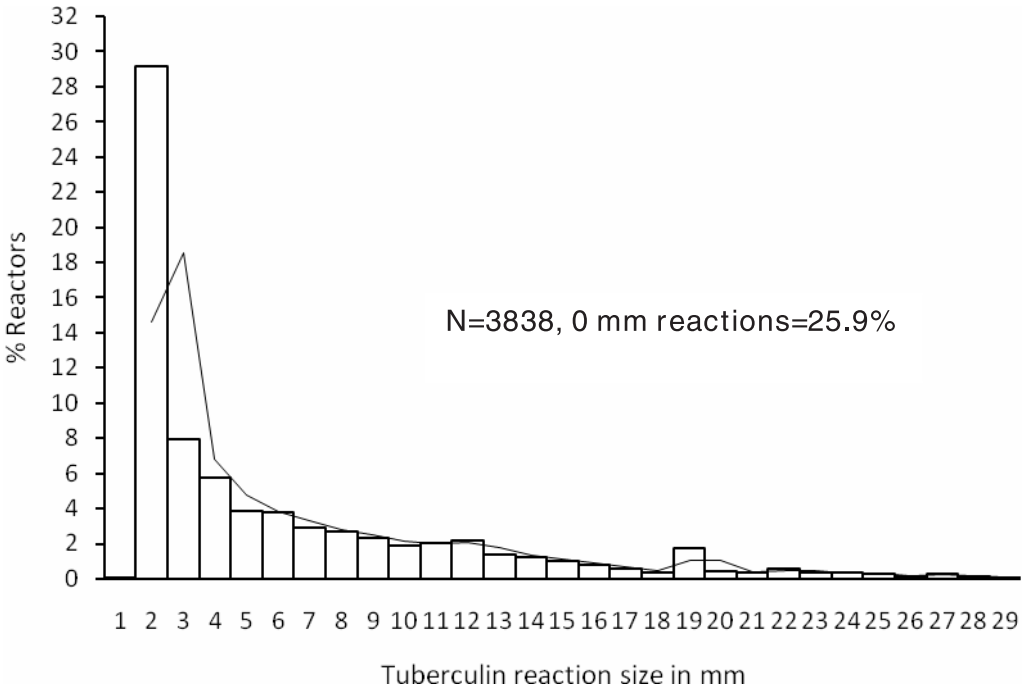

Figure 1: Frequency distribution of tuberculin reaction sizes-all children

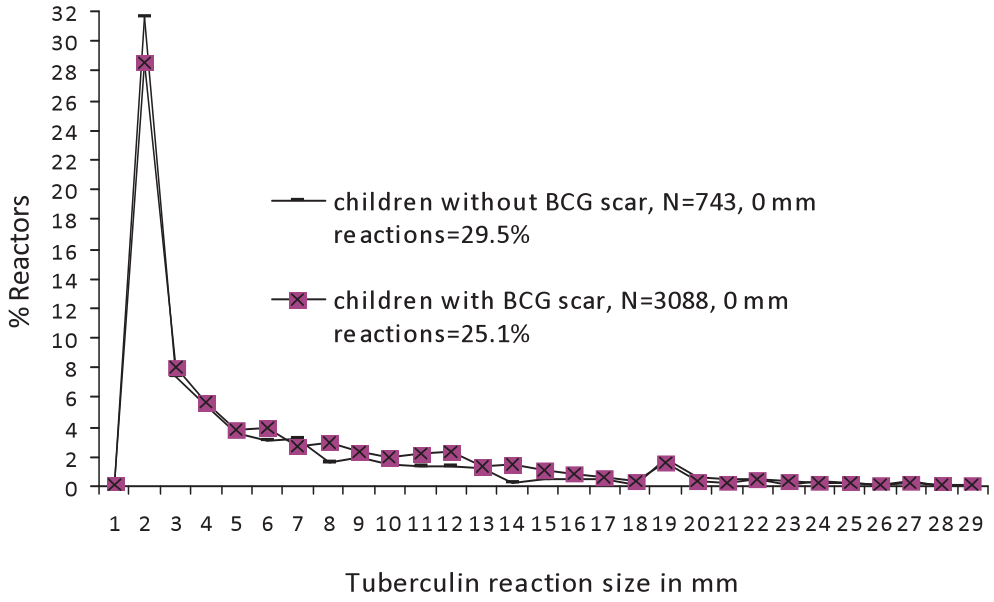

Figure 2: Frequency distribution of tuberculin reaction sizes by BCG scar status

prevalence of smear positive PTB at 108 (CI: 82-134) among persons above 14 years of age, one per cent ARTI corresponded to a prevalence of 103 sputum smear positive patients of PTB {range 78-128, based on lower and upper limits of prevalence of smear positive TB and a fixed ARTI of 1.05%}.

DISCUSSION

The present survey was carried out among children 5–9 years of age. Children irrespective of the presence or absence of BCG scar were included in the survey. That inclusion of children vaccinated with BCG as per Universal Immunization Programme (UIP) in the survey does not affect ARTI estimates has been demonstrated in many studies in India[8,9]. Moreover, the BCG induced tuberculin sensitivity wanes significantly by five years of age when children are vaccinated during early infancy[10,11]. The age group of 5-9 years was also considered as the most appropriate since a high prevalence of infection with non-tuberculous mycobacteria (NTM) in higher age groups could compound the challenges in interpretation of survey data[12].

Prevalence of infection was estimated by mirror–image technique which is based on the assumption that reactions due to infection with tubercle bacilli are distributed normally around the second mode. Since BCG induced reactions are generally smaller than this mode, influence of BCG-induced tuberculin sensitivity on estimate of prevalence of infection by mirror image technique is minimal. This was also observed in the present study as the estimated ARTI rates were similar between children without and with BCG scar. The ARTI estimated by anti-mode method among children without BCG scar was also similar. The anti-mode method was not applied to all children since a significant proportion of BCG induced reactions may be distributed around the anti-mode.

The ARTI among all children was estimated at 1.05% in the present study. The mode used for estimation by mirror image technique seems robust since similar modes at 19 mm were also observed among children during first southern zonal tuberculin survey in 2000-2001 and confirmed TB patients during the second zonal level survey in 2009-10 in six districts of the southern zone of the country[4]. ARTI estimate for the entire southern zone during second zonal survey was similar at 1.0%[4].

While the estimated ARTI applied to the year 2008, TB disease survey was carried out with the mid-point of the survey period in 2009. Thus in the present study area, one percent of ARTI corresponded to 103 sputum smear positive patients of PTB per 100 000 population (> 14 years of age). It was similar to the prevalence of 106 for every one percent ARTI observed during the pre-DOTS period in the same district and within the range of 80-120 observed by Styblo in other countries during the pre-chemotherapy and pre-DOTS era[1].

Considering the ARTI of 1.05%, 1050 individuals out of every 100,000 population get newly infected with tubercle bacilli every year. The prevalence at 103 per 100,000 population implies that each adult point prevalence case of smear positive pulmonary TB infects about 10 children per year. This was similar to the observation made in the same study area during pre-DOTS period[1]. This scenario of no change despite increase in treatment success rates may be attributed to delay in case finding and increase in the average number of susceptible contacts per case due to increase in population density and greater mobility. In Chingleput / Thiruvallur, this ratio was 10 during pre-DOTS period and eight in DOTS period[1,2]. Lower numbers of infections per prevalent case have been observed in China, Korea and Phillipines which could be due to differences in case-contact ratios[13]. This indicates that the relationship between ARTI and prevalence of smear positive PTB observed in one area/region cannot be applied to other areas and thus precludes estimating prevalence from ARTI estimates.

In conclusion, the results of the present study suggest the need for early case detection and treatment in order to reduce the number of infections caused per patient and bring down the pool of infected persons in the community.

ACKNOWLEDGEMENTS

The authors are thankful to the children who participated in the survey and their parents and school teachers. Thanks are also due to all the officials of the National TB Institute involved in field work and data entry.

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TUBERCULAR ILEAL PERFORATION - ATYPICAL, ACUTE PRESENTATION IN A RENAL TRANSPLANT RECIPIENT - A CASE REPORT

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(Received on 6.9.2012; Accepted after revision on 31.5.2013)

Summary: Extrapulmonary tuberculosis (TB) is more common than pulmonary TB in immuno-suppressed renal transplant recipients. Atypical presentation of TB and disseminated TB is known in transplant recipients. Usually intestinal TB presents with pain abdomen, intermittent subacute intestinal obstruction, diarrhoea and/or constitutional symptoms like fever and weight loss. Here we report a case of renal allograft recipient on regular hospital follow up, presented with acute abdomen with no previous symptoms of fever, weight loss or abdominal symptoms and was diagnosed to have tubercular ileal perforation on exploratory laporatomy and confirmed by histopathological examination. This patient succumbed to the illness due to sepsis despite timely surgery, broad spectrum antibiotics and antitubercular therapy. **[*Indian J Tuberc 2013; 60:* 233-236]**

Key words: Renal transplant, Extrapulmonary TB, Ileal perforation

INTRODUCTION

Tuberculosis is responsible for significant morbidity and also mortality in renal transplant recipients in developing countries.[1] Often, tuberculosis in renal transplant recipients is disseminated and extrapulmonary. Defective cell mediated immunity due to cumulative effects of immuno-suppressive antirejection drugs in transplant recipients favours tuberculosis.[2] Also use of immuno-suppressive agents masks the inflammatory response and hence manifestations of infection like fever, pain at site of tissue injury are masked. Hence clinical manifestations of pulmonary or extra-pulmonary TB are atypical.[3]

Here, we report a young renal transplant recipient, three years post transplant with stable renal functions, presenting acutely with pain abdomen with no previous symptoms like fever, altered bowel habits, past pain abdomen, decreased appetite and an urgent exploratory laprotomy revealed 'ileal perforation', multiple tubercules on intestinal surface and multiple mesentric matted lymphnodes. Tuberculosis was confirmed by histopathological examination of surgical specimen.

CASE REPORT

A 38-year-old male patient received a renal allograft from his 58-year-old mother with full '6 antigen' HLA match in 2008. His native kidney disease was presumed chronic glomerulonephritis and he was on maintenance hemodialysis for six months before renal transplantation. He was negative for Hepatitis B, Hepatitis C and HIV virus and both donors and recipients were CMV IgG positive. He was on triple immuno-suppression, Tacrolimus, Mycophenolate mofetil and prednisolone. His post surgical period was uneventful and he reached a nadir serum creatinine of 1.1mg/dl on third post operative day.

Eleven months later, patient had herpes zoster of right side D4 and D5 dermatome, which was managed with Tab. Acyclovir and also reduction in immuno-suppressive drug mycophenolate mofetil. One and half years post transplant, patient had an episode of acute cellular rejection (Banff Ib) during an attempt at calcineurin inhibitor withdrawal.

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Patient's creatinine increased to 2.8mg/dl from baseline of 1.2mg/dl. Patient was pulsed with three doses of intravenous pulse methylprednisolone 500mg each and tacrolimus was reintroduced. His graft function recovered partially and patient's creatinine settled at 2.0mg/dl. A graft biopsy six weeks later showed features of chronic allograft nephropathy. For the next one and half years, patient was stable with s. creatinine around 2.1 – 2.2mg/dl. He was on regular follow up once in three months and he was on Tacrolimus 1mg twice daily, Mycophenolate 750mg twice daily and prednisolone 10mg/day.

During one of the follow up visits, three years post transplant, patient complained of pain in the gluteal region and he was found to have a small, fluctuating gluteal abscess and was admitted for incision and drainage of the same. Pus from the abscess showed staphylococcus aureus and was treated with intravenous amoxycillin + clavulanic acid as per culture and sensitivity.

Two days later during hospital stay, patient had sudden onset of colicky pain abdomen and vomiting following defecation. On clinical evaluation, patient was hemodynamically stable and afebrile. He had diffuse abdominal tenderness and minimal guarding, sluggish bowel sounds. A clinical suspicion of acute pancreatitis / hollow viscous perforation was kept and a complete work up for acute abdomen was done. Serum amylase and lipase were normal. Erect x-ray abdomen showed gas under both the diaphragms and CT abdomen (without contrast) also showed presence of subdiaphragmatic gas.

Patient underwent urgent exploratory laparotomy and at laparotomy a '5x3'cms ileal perforation was seen around 20cm from ileocecal junction. The external surface of ileum showed multiple small nodules (tubercles) and multiple enlarged mesentic nodes were seen. The lymphnodes on sectioning showed caseous material. Ileal resection with end-to-end anastomosis was done.

At histopathology, chronic transmural chronic inflammatory infiltration of the resected ileum consisting of lymphocytes, sheets of macrophages and multinucleated giant cells with a few neutrophils was seen. There were granulomas in the mesentry. The mesentric lymphnodes showed numerous acid fast bacilli on ZN staining suggestive of *Mycobacterium tuberculosis* (Figures 1-3).

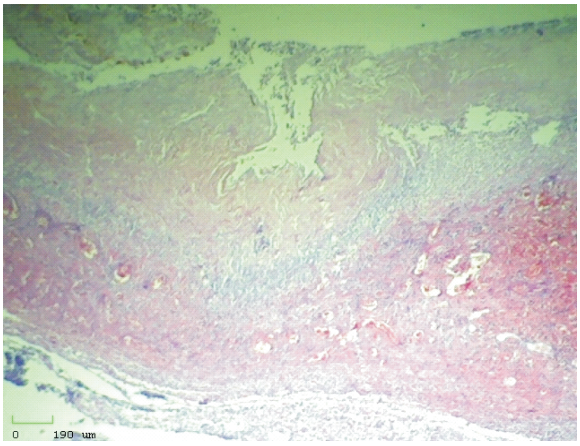

Fig. 1: Wall of small intestine showing transmural necrosis with a dense inflammatory infiltrate. (5x, H&E stain)

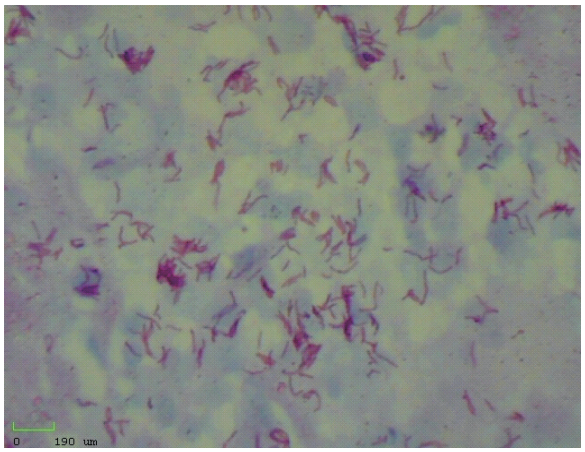

Fig. 2: Numerous acid fast bacilli in lymphnodes (100x, ZN stain)

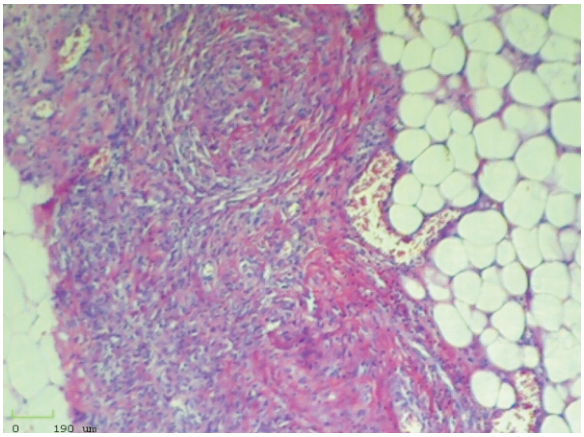

Fig. 3: Ill formed granulomata seen in the mesentry. (10x, H&E stain)

Patient was managed in intensive care unit and was weaned off ventilator on day 2. Patient was kept nil by mouth and was on IV piperacillin plus tazobactum, IV metronidazole. Also IV ciprofloxacin 400mg per day and IV Amikacin 750mg per day was started as initial antitubercular agents with an intent to change over to oral antitubercular agents, when patient is allowed orally. Patient's s. creatinine had increased to 3mg/dl, but patient had good urine output and was hemodynamically stable. Immuno-suppression was reduced to mycophenolate mofetil 500mg twice daily and injection hydrocortisone 100mg IV thrice daily, tacrolimus was withdrawn.

Patient started having high fever from day 3 post op. and soon had hypotension, bilateral lung crackles. He was intubated and ventilated, started on noradrenaline support and antibiotics escalated to IV meropenem and also empirical IV fluconazole was started. The abdomen was soft and drain showed no increased collections. His inotrope requirement to maintain blood pressure progressively increased and patient succumbed to refractory septic shock on post operative day 5. Relatives declined a post-mortem evaluation.

DISCUSSION

The prevalence of post renal transplant tuberculosis is 3.1 to 15% in Asia, 1.5 to 8.5% in South Africa, 1.5 to 3.5% in Middle East, 1.7 to 5% in Europe and 1.5% in United States.[4] Extrapulmonary tuberculosis is more common than pulmonary tuberculosis in immuno-suppressed solid organ transplant recipients as there is failure to inhibit spread of tuberculosis by lymphohaematogenous route. Renal allograft recipients commonly present with extrapulmonary tuberculosis (51.3%)[2] and prevalence of gastrointestinal tuberculosis (GITB) among renal transplant recipients varied from 0.6 to 3%.[5, 6]

About 45-60% of tuberculosis occurs in the first year after transplantation. The median time for onset of TB is about nine months post transplantation.[4] In the Indian scenario, the median onset of post transplant TB is 26 months in patients who received azathioprine and prednisolone as immuno-suppression and 11 months in those who were on cyclosporine along with azathioprine and prednisolone.[7] An early occurrence was noted with non-renal solid organ transplantation, anti CD3 therapy, malnutrition secondary to long dialysis vintage.[8] Immuno-suppression with tacrolimus or mycophenolate has also been associated with earlier development of TB post transplantation.[9]

The common presenting symptoms of GITB in non-transplant patients are abdominal pain, constipation/diarrhoea/weight loss and fever. In transplant recipients, the presentation may be atyptical, acute presentation and also GITB is usually a part of disseminated tuberculosis.[10] Usually the picture may be chronic with pyrexia of unknown origin or vague symptoms of weight loss and abdominal pain of a few days or weeks, and diagnosis is often delayed due to non-specific nature of illness requiring multiple imaging procedures.[11]

In transplant recipients, the use of immuno-suppression masks the symptoms of fever and even reduces pain due to inflammation, possibly decreased inflammation of 'peritoneum' in this case scenario which is the pain causing structure in abdominal koch's. Our patient did not have any constitutional symptoms like fever, weight loss, chronic abdominal pain and presented acutely with pain abdomen and

ileal perforation. Also due to use of immuno-suppression, even hollow viscous perforation signs like abdominal guarding and rigidity were minimal. Ileocecal and jejunoileal regions are the most common sites of involvement in GITB.[4] Malabsorption, obstruction and diarrhoea, ileocecal mass are common modes of presentation. TB accounts for 5.9% of all small intestinal perforation in India and is second to enteric fever in causing intestinal perforation.[12] GITB in a transplant scenario is more likely to present with ulcerative lesions and perforation than in non-transplant scenario, as immuno-suppression including steroids induce less fibrotic elements during attempts at healing process. Even caecal perforation has been reported earlier.[13]

CONCLUSION

Gastrointestinal tuberculosis may present atypically, with minimal symptoms in immuno-suppressed solid organ transplant recipients. Immuno-suppressive drugs may mask the constitutional and local signs and symptoms of inflammation or infection. **It is important to know the varied presentations in the transplant scenario for enhancing clinical suspicion and making early diagnosis. Perhaps tubercular abdomen should be considered in the differential diagnosis of acute abdomen in transplant recipients in developing countries like India where TB is endemic**.

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Case Report

GALLBLADDER TUBERCULOSIS MIMICKING MALIGNANCY: A RARE CASE REPORT

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(Received on 3.10.2012; Accepted after revision on 1.6.2013)

Summary: Gallbladder tuberculosis is an extremely rare disorder even in endemic region. It often mimics gallbladder malignancy as both of them share some common presentations. This entity is very rarely diagnosed pre-operatively as neither clinical features nor radiology are pathognomonic of gallbladder tuberculosis. The case reported here presented as chronic calculous cholecystitis with mass at gallbladder neck. Patient underwent laparotomy with suspicion of gallbladder carcinoma, which was eventually diagnosed as a case of gallbladder TB following histopathological examination of the resected specimen. He also had pulmonary TB in association and was completely cured with short course antitubercular chemotherapy. **[*Indian J Tuberc 2013; 60:* 237-240]**

Key words: Gallbladder Tuberculosis, Gallbladder Malignancy, Chronic Cholecystitis.

INTRODUCTION

Abdominal tuberculosis is common in developing countries but gallbladder tuberculosis is a rare entity and only a few cases have been found in literature. The first case of tuberculosis of gallbladder in the world literature was described in 1870 by Gaucher[1]. Gallbladder TB often mimics gallbladder carcinoma in patients presenting with gallbladder mass. And due to lack of pathognomonic features on radiology, this is diagnosed mostly after histopathological examination of resected specimen. Here we report a case of gallbladder TB with pulmonary involvement which was initially suspected to be a case of gallbladder malignancy.

CLINICAL RECORD

A 39-year-old male patient, presented with complaints of dull-aching pain over mid and right upper abdomen and fever since two months. The pain was of moderate intensity, localized, increased after taking meal and relieved by anti-spasmodic medication. The fever was intermittent, high grade, without any chill, rigor or diurnal variation. There was no history of vomiting, yellow discolouration of eyes and urine, altered bowel habit, heartburn, haematemesis or bleeding per rectum. He denied any history of cough, expectoration, dyspnoea or chest pain. There was past history of peptic ulcer disease 18 years back for which he was treated conservatively. Prior history of tuberculosis or its contact was absent. He was farmer by occupation, non-smoker but addicted to alcohol since more than 10 years.

On examination, patient was of average built, febrile with heart rate 106/minute. Pallor, icterus, oedema or palpable lymphadenopathy were all absent. Abdominal examination revealed presence of mild epigastric and right hypochondriac tenderness without any palpable mass or organomegaly. Bowel sound was audible. No clinical evidence of ascites was present. Examination of respiratory, cardiovascular and nervous system was normal.

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Routine blood investigation showed Hb-13 gm/dl, total leucocyte count 8200/mm^3 with polymorph 68%, Lymphocyte 26% and eosinophil 6%. Fasting blood sugar, serum urea, creatinine, liver function test and ECG were normal. Serology was negative for HIV, HBsAg and HCV. Stool for occult blood was negative. USG of abdomen revealed presence of a hypoechoic SOL (2.6cm X 2.9cm) at the neck of the gallbladder compressing proximal common bile duct with a gallbladder calculous and associated thickening of gallbladder wall suggestive of chronic cholecystitis. An upper GI endoscopy found a partially healed chronic anterior bulbar duodenal ulcer with severe erosive gastritis. On CECT abdomen, gallbladder was found to be distended with thickened wall and a calculous in neck region, suggesting a diagnosis of chronic cholecystitis and cholelithiasis (Fig. 1). Pre-operative chest X-ray was normal. A decision of surgery was taken considering possibility of gallbladder malignancy although the differential diagnosis of chronic calculous cholecystitis was kept in mind.

Patient had undergone laparotomy with right subcostal incision under general anaesthesia. On exploration, gallbladder was found to be thick-walled with a nodular mass at the neck adhered to liver. There were nodules at porta hepatis and first part of duodenum. Partial cholecystectomy was done and tissue was sent for histopathological examination which showed features of chronic granulomatous lesion with caseating granuloma,

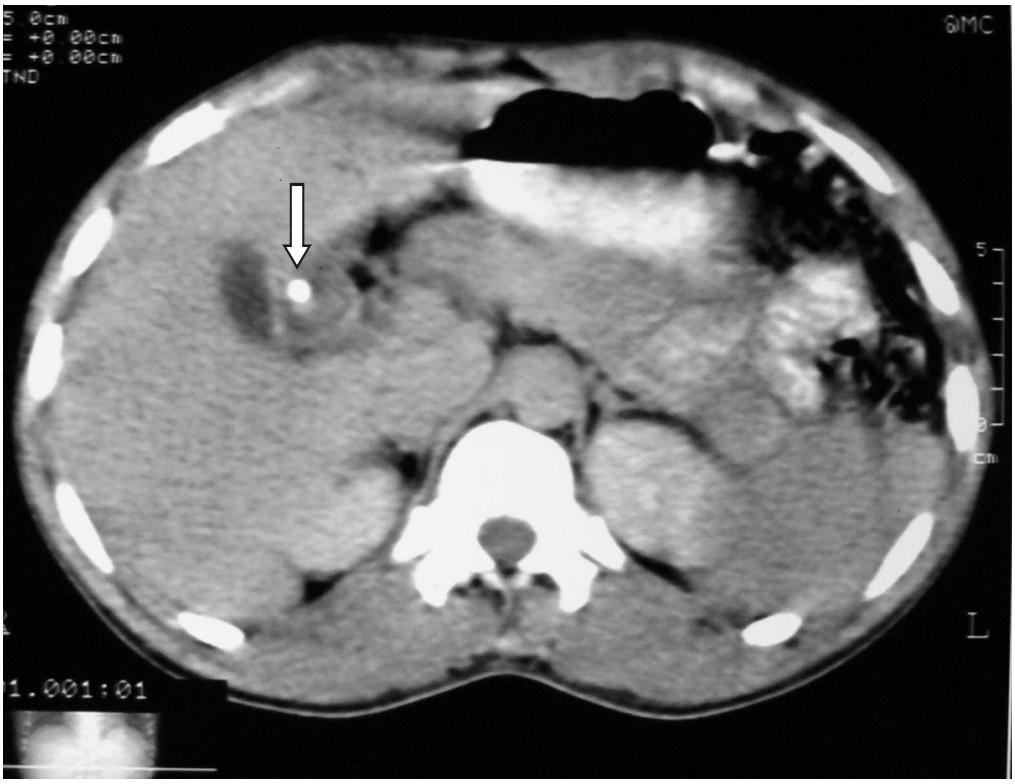

Fig. 1: CECT abdomen showing rounded hyperdense area at neck region of gallbladder suggestive of calculi along with thickened gallbladder wall

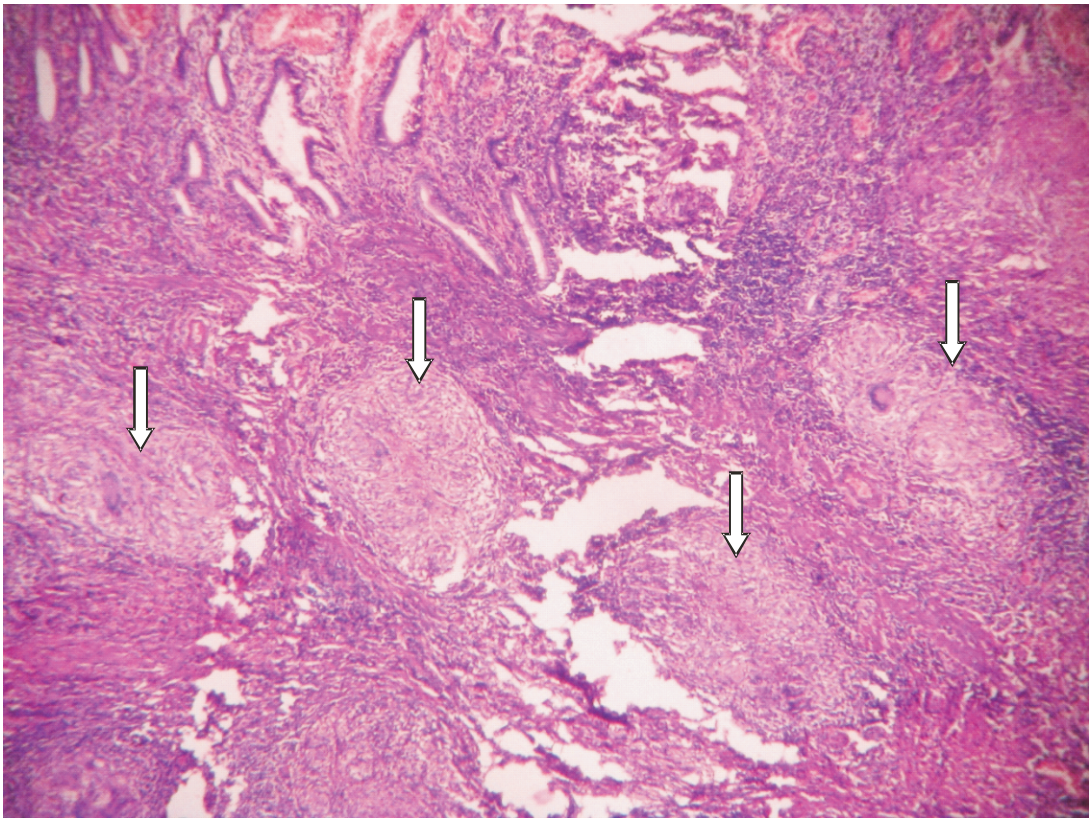

Fig. 2: Histopathology of resected gallbladder specimen showing confluent granuloma (indicated by arrows) in submucosal layer along with multinucleate giant cells (Hematoxylin & eosin; 100X)

multinucleate giant cells and epithelioid cells in submucosal layer (Fig. 2). Post-operative chest X-ray showed presence of infiltrative lesions in right upper zone. His sputum samples were negative for AFB. Mantoux test with 5TU produced an induration of 18mm and serum ADA was elevated with a value of 65 IU/L.

He was discharged with anti-tubercular therapy category I under RNTCP as a case of sputum negative pulmonary TB with TB gallbladder. Patient symptomatically improved with therapy and chest X-ray lesion also disappeared during the course of treatment.

DISCUSSION

Hepatobiliary tuberculosis is rare and is seen in approximately 1% of all abdominal tuberculosis cases[2]. It occurs most commonly in women over 30 years of age[3]. The normal gallbladder has an unusually high resistance to tubercular infection due to presence of inhibitory factors in bile[2,4]. The presence of an underlying pathology in the form of cholelithiasis or cystic duct obstruction is said to be essential for the development of gallbladder tuberculosis[2] as we found in our case. Route of infection can be lymphatic (adjacent caseating lymph nodes), canalicular (ascending directly through an inflammed bile duct) or haematogenous.

Four distinct varieties of gallbladder tuberculosis are recognised[5] : (a)as a component of miliary TB in children and in adults, (b)as a component of disseminated abdominal TB[5],

(c)isolated gallbladder TB without overt tubercular foci elsewhere in the body, (d)involvement of GB in anergic states due to uraemia[5], cancer[6] or AIDS. Though majority of patients belong to third group variant, that was not the situation in our case where pulmonary involvement was present simultaneously. Concomitant pulmonary TB was seen in 28.9% cases of gallbladder TB in an Indian study[7] whereas in world literature it varied from 10% to as high as 65%. So, it is mandatory to search for other foci of tuberculosis, especially in lung in such cases.

There is no pathognomonic presentation of this condition and can vary from non-specific symptoms of fever, anorexia, abdominal pain, jaundice to even gallbladder perforation. An unusual presentation of a persistent port-site sinus in a patient after laparoscopic cholecystectomy (due to cholelithiasis) has also been recorded[8]. Imaging findings, though often, describe features of chronic cholecystitis and cholelithiasis or intraluminal mass lesion, such findings are non-specific and a histological confirmation is absolutely essential. Positive yield of AFB from bile cytology on ERCP is also very low, further complicating the diagnosis[9]. Although serum ADA is not routinely measured, when elevated, it may support tubercular aetiology[10]. The differential diagnosis of gallbladder tuberculosis includes acute and chronic cholecystitis, gallbladder carcinoma[3] (which was the first impression during initial evaluation in above case) and polypoid lesions. Treatment protocol being the same to other form of abdominal TB, consists of four-drug regimen during intensive phase and two-drug regimen during continuation phase.

Finally, it can be concluded that **it is important to have a high index of suspicion of tuberculosis in such patients, particularly in endemic region, even if their clinical diagnosis shows a case of chronic cholecystitis or malignancy, to avoid unnecessary delay in institution of appropriate therapy**.

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Case Report

PRIMARY TUBERCULOUS MYOSITIS: A RARE CLINICAL ENTITY

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(Received on 24.1.2013; Accepted after revision on 8.6.2013)

Summary: Primary tuberculous myositis without underlying pathology has been sparingly reported in medical literature. We report a case of primary tuberculous myositis of left upper arm in a seven-year-old boy. He presented with gradually increasing swelling on the medial aspect of the left arm. Ziehl Neelsen staining of pus collected revealed acid fast bacilli morphologically resembling M*ycobacterium tuberculosis* and the same was grown on the culture. Histopathological findings were consistent with tuberculosis. The results were confirmed by Genotype MTBDR*pluse* line probe assay. He was treated with standard four-drug regimen to which he responded well with complete resolution of the lesion. **[*Indian J Tuberc 2013; 60:* 241-244]**

Key words: Tuberculous myositis, *M. tuberculosis*

INTRODUCTION

Tuberculosis is one of the major diseases affecting children worldwide. It causes a significant morbidity and mortality, especially in infants and young children. In developing countries, the annual risk of tuberculosis in children is 2-5% and in India it is 1.5%.[1] Though lung is the commonest site of infection, extra-pulmonary tuberculosis is seen in 2.5% of cases. About 3% of patients with extra-pulmonary tuberculosis have musculoskeletal involvement in the form of spondylitis, osteomyelitis or arthritis.[2] Involvement of skeletal muscle without co-existing active disease is very rare. The incidence of primary muscular tuberculosis was reported as 0.015% by Petter.[3] Early diagnosis is often missed because of the infrequent occurrence and non-specific clinical manifestations which leads to delay in treatment resulting in irreversible limb deformity and functional disability.[4]

We report a case of primary tuberculous myositis of left arm in a seven-year-old boy with no obvious underlying bone lesion.

CASE REPORT

An apparently healthy seven-year-old boy presented with gradually increasing swelling on the medial aspect of the left upper arm since last 15 days. There were no other systemic symptoms. There was no history of trauma, intramuscular injection, family history of tuberculosis or exposure to any known person with active tuberculosis. Local examination revealed cystic swelling 5 cm × 4 cm × 4 cm in size on medial aspect of the left arm. It was slightly tender, well separated from the underlying bone and was not fixed to the skin or deeper tissue. The skin over it was normal (Figure 1). There was no regional lymphadenopathy. Patient had received national immunization schedule successfully. Clinical diagnosis of hematoma or lipoma was made and fine needle aspiration was done. Aspirate was sent for cytology and microbiological analysis. Patient was put on antibiotics and was advised to visit after 15 days. Cytology of aspirate revealed numerous acute inflammatory cells and histiocytes. Gram stain showed a few pus cells and no organisms. Ziehl Neelsen (ZN) stain was positive for acid fast bacilli. Aerobic and anaerobic cultures were sterile. Sample was

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Figure 1: Swelling on the medial aspect of left upper arm.

inoculated on Lowenstein Jensen medium. After three weeks, patient revisited with persistence of swelling and low grade fever and was admitted for incision and drainage.

His hemoglobin was 11.7gm%, WBC count 11300/cmm with 54% polymorphs, 34% lymphocytes, 6% monocytes, 4% eosinophils and 2% basophils. ESR was 28 mm at the end of one hour by wintrobes method. Blood sugar, urine analysis and renal function tests were within normal limits. Patient was seronegative for HIV by rapid and ELISA test. HBsAg test was negative. Mantoux test was strongly positive with the induration measuring 19 mm at 48 hours.

Ultra sound scan of the swelling showed an oblong lesion measuring 35 × 18.5 mm. The lesion showed encysted echogenic fluid with small mural lesion of size 15.8 × 10.3 mm in superficial layer of muscle (Figure 2A). On Colour Doppler, there was

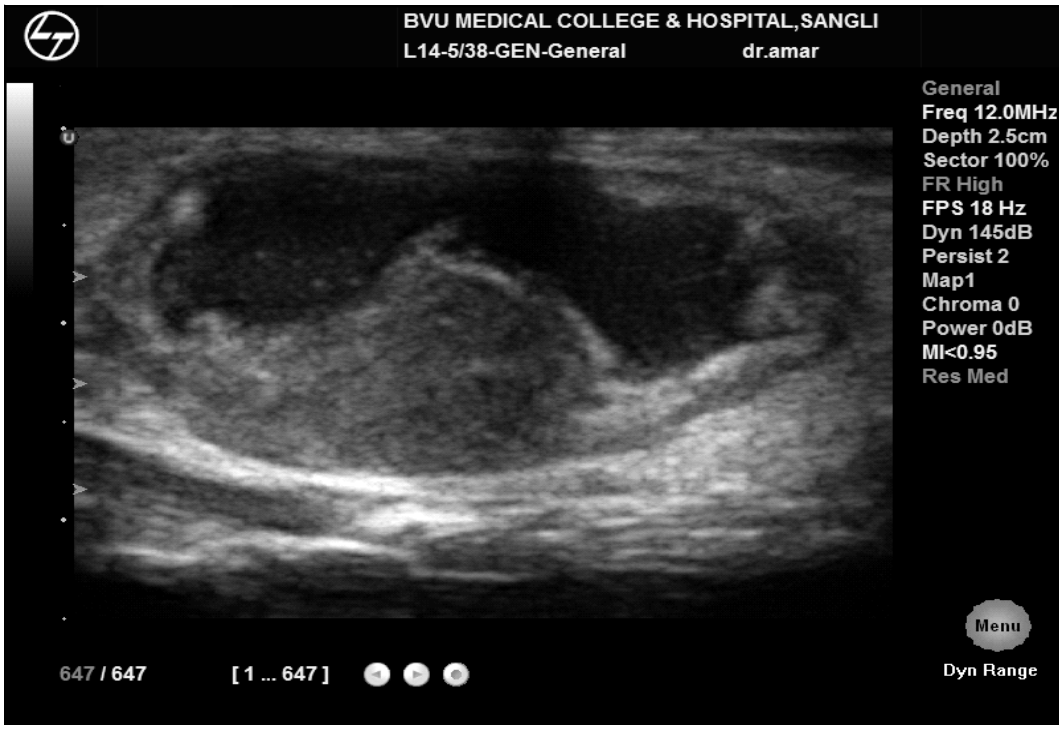

Figure 2A:- USG of upper arm showing encysted echogenic fluid in the superficial layer of muscle.

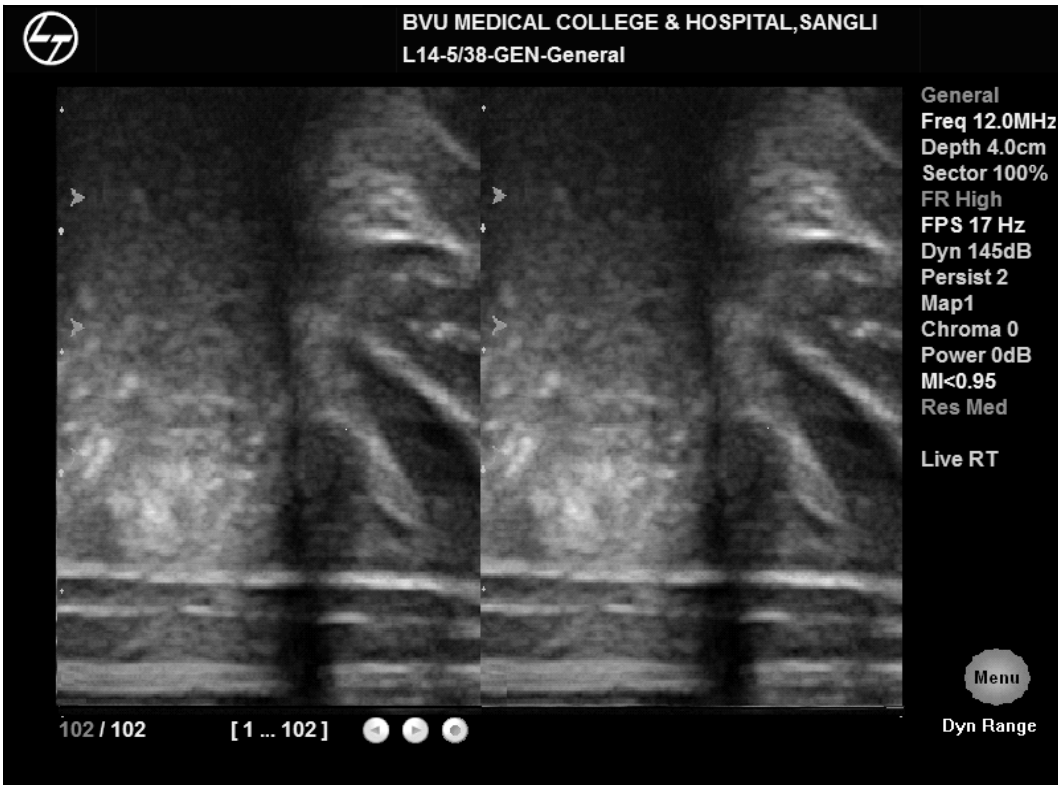

Figure 2B:- Follow up USG of upper arm showing resolution of lesion.

no evidence of increased vascularity in the lesion. There was no evidence of involvement of bone. Ultrasonography of abdomen and chest X-ray was normal. Under general anesthesia, the lesion was excised and 3-4 ml of pus was drained along with abscess wall and was sent for microbiological and histopathological examinations.

Gram and ZN staining of pus revealed similar findings as those of previous one. Aerobic and anaerobic culture was sterile. However, both the samples yielded growth of slowly growing mycobacteria on LJ medium. The isolate was identified as *Mycobacterium tuberculosis* by standard methods.[5] Antibiotic sensitivity testing was carried out by proportion method.[6] Isolate was found to be sensitive to Rifampicin, Isoniazid, Pyrazinamide, Ethambutol and Streptomycin. These findings were confirmed by GenoType MTBDR*pluse* line probe assay method (Hain Lifescience GmbH, Nehren, Germany) targeting *rpoB, katG* and *inhA* genes.

Haematoxlin and eosin stain of excised granulation tissue revealed, fibrocollagenous tissue with area of caseous necrosis, epithelioid cells, Langhan's giant cells and lymphocytes suggestive of tuberculosis.

The patient was referred to DOTS and was given four-drug antitubercular chemotherapy to which he responded well. Repeat USG of the left upper arm after six months showed complete resolution of the lesion (Figure 2B).

DISCUSSION

Tuberculosis of the soft tissue without obvious underlying pathology is extremely rare. The skeletal muscles are rarely affected in tuberculosis because they are not favourable site for the survival and multiplication of *Mycobacterium tuberculosis*. This has been attributed to poor oxygen, high lactic acid content, absence of reticuloendothelial or

lymphatic tissue, rich blood supply and highly differentiated state of muscle tissue.[7] Tuberculosis can involve skeletal muscles by extension from bone, synovial lining of joints, tendon sheaths, by direct inoculation (trauma, syringe), or rarely by hematogenous dissemination.[8] However, selective primary involvement is extremely rare.

Tuberculous myositis is frequently misdiagnosed as sarcoma, soft tissue tumour, parasitic infections like cysticercosis or hydatid cyst, fungal infection, hematoma or lipoma.[4] The infection is restricted to one muscle but several muscles may be involved. There may be frank abscess as seen in the present case or nodular sclerosis followed by calcification.[8] Due to atypical presentation, lack of early signs, diagnosis is often delayed resulting in widespread involvement, atrophy and deformity of the affected part. The tuberculous involvement of the left arm muscle in the present case seems to be primary because there was no evidence of tuberculous foci elsewhere in the body. It may be from an occult primary focus somewhere. In the present case, outcome was excellent after surgical intervention and appropriate antitubercular therapy.

Molecular techniques like PCR or GenoType MTBDR*pluse* line probe assay are tools for rapid diagnosis. However, culture and histopathology remain the gold standard. **A normal chest radiograph, absence of systemic symptoms or absence of other focus of active tuberculosis should not dissuade one from making diagnosis. High index of suspicion is needed for early diagnosis and treatment in endemic areas like India**.

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ABSTRACTS

Association between *Mycobacterium tuberculosis* lineage and time to sputum culture conversion

E.S. Click, C.A. Winston, J.E. Oeltmann, P.K. Moonan, and W.R. Mac Kenzie. *The International Journal of Tuberculosis and Lung Disease* 2013; **17(7)**: 878-84.

Mycobacterium tuberculosis comprises four principal genetic lineages: one evolutionarily ancestral (Indo-Oceanic) and three modern. Whether response to tuberculosis (TB) treatment differs among the lineages is unknown. The objective of the study was to examine the association between *M. tuberculosis* lineage and time to sputum culture conversion in response to standard first-line drug therapy. We conducted an exploratory retrospective cohort analysis of time to sputum culture conversion among pulmonary tuberculosis (PTB) cases reported in the United States from 2004 to 2007. The analysis included 13,170 PTB cases with no documented resistance to first-line drugs who received a standard four-drug treatment regimen. Among cases with baseline positive sputum smear results, relative to cases with Euro-American lineage, cases with Indo-Oceanic lineage had higher adjusted hazards of sputum culture conversion (aHR 1.32, 95%CI 1.20-1.45), whereas cases with East-African-Indian or East-Asian lineage did not differ (aHR 1.05, 95%CI 0.88-1.25 and aHR 0.99, 95%CI 0.91-1.07, respectively). Among cases with baseline negative sputum smear results, time to sputum culture conversion did not differ by lineage. Although these results are exploratory, they suggest that the eradication of viable bacteria may occur sooner among cases with Indo-Oceanic lineage than among those with one of the three modern lineages. Prospective studies of time to sputum culture conversion by lineage are required.

Nicotine Treatment Improves Toll-Like Receptor 2 and Toll-Like Receptor 9 Responsiveness in Active Pulmonary Sarcoidosis

Mark W. Julian, Guohong Shao, Larry S. Schlesinger, Qin Huang, David G. Cosmar, Nitin Y. Bhatt, Daniel A. Culver, Robert P. Baughman, Karen L. Wood and Elliott D. Crouser. *Chest* 2013; **143(2)**: 461-70.

New evidence links nicotine to the regulation of T cell-mediated inflammation via Ü7 nicotinic cholinergic receptor activation, and chronic nicotine exposure (smoking) reduces the incidence of granulomatous diseases. We sought to determine whether nicotine treatment was well tolerated while effectively normalizing immune responses in patients with active pulmonary sarcoidosis. Consented adults with symptomatic sarcoidosis (n = 13) were randomly assigned to receive 12 weeks of nicotine treatment plus conventional therapy or conventional therapy alone. Obtained blood cells were evaluated for their responsiveness to selected Toll-like receptor (TLR) and nucleotide oligomerization domain-like receptor ligands and T cell surface marker expression before and after nicotine treatment. Asymptomatic patients (n = 6) and disease-free subjects (n = 6) served as comparative control subjects. Adverse events were monitored for the duration of the study. Compared with the asymptomatic group, symptomatic patients had impaired peripheral responses to TLR2, TLR4, and TLR9 ligands (anergy) and reduced peripheral populations of CD4+ FoxP3+ regulatory T cells (Tregs). Nicotine treatment was associated with restoration of TLR2 and TLR9 responsiveness, and expansion of Tregs, including the CD4+CD25 FoxP3+ phenotype. There were no serious adverse events or signs of nicotine dependency. Nicotine treatment in active pulmonary sarcoidosis was well tolerated and restored peripheral immune responsiveness to TLR2 and TLR9 agonists and expansion of FoxP3+ Tregs, including a specific "preactivated" (CD25) phenotype. The immune phenotype of patients with symptomatic

sarcoidosis treated with nicotine closely resembled that of asymptomatic patients, supporting the notion that nicotine treatment may be beneficial in this patient population.

Isoniazid preventive treatment: predictors of adverse events and treatment completion

L.R. Codecasa, N. Murgia, M. Ferrarese, M. Delmastro, A.C. Repossi, L. Casali, G. Besozzi, G. Ferrara, and M.C. Raviglione. *The International Journal of Tuberculosis and Lung Disease* 2013; **17(7)**: 903-8.

The study was conducted at the Villa Marelli Institute (VMI), Niguarda Ca'Granda Hospital, Milan, Italy. A recent report on the fatal side effects of isoniazid preventive therapy (IPT) from the United States has re-ignited discussion on the safety of this intervention. The aim was to evaluate IPT feasibility, treatment completion and adverse events (AE) and their determinants under field conditions. Data from consecutive subjects undergoing IPT at the VMI were recorded in an electronic database from 1992 to 2009. Logistic regression analysis was performed to detect completion and AE determinants. A total of 11, 963 patients were included in the study. AE (odds ratio [OR] 2.70, 95%CI 2.22-3.28) and human immunodeficiency virus positive status (OR 5.20, 95% CI 2.10-12.93) were the main determinants of treatment interruption among Italians, while social weakness (no housing/job; OR 2.88, 95%CI 2.43-3.42), AEs (OR 1.33, 95% CI 1.15-1.53, 2.22-3.28) and screening in undocumented subjects (OR 1.20, 95% CI 1.01-1.44) prevailed among foreigners. Age was the main determinant of transaminase increase (OR 1.03, 95% CI 1.03-1.04), as were AEs of the gastrointestinal (OR 1.02, 95%CI 1.02-1.03), central nervous (OR 1.02, 95%CI 1.02-1.05) and peripheral nervous systems (OR 1.04, 95% CI 1.02-1.05). This analysis demonstrates the feasibility and safety of IPT, with determinants of interruption and AEs being predictable and addressable.

Diagnostic accuracy of Xpert® MTB/RIF on bronchoscopy specimens in patients with suspected pulmonary tuberculosis

H.Y. Lee, M.W. Seong, S.S. Park, S.S. Hwang, J. Lee, Y.S. Park, C.H. Lee, S.M. Lee, C.G. Yoo, Y.W. Kim, S.K. Han, and J.J. Yim. *The International Journal of Tuberculosis and Lung Disease* 2013; **17(7)**: 917-21.

The objective of the study was to determine the diagnostic accuracy of the Xpert® MTB/RIF assay using samples obtained through bronchoscopy in patients with suspected pulmonary tuberculosis (PTB). We retrospectively reviewed the records of patients with suspected PTB for whom the Xpert MTB/RIF assay was performed on bronchoscopy specimens. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the diagnosis of active PTB were calculated for acid-fast bacilli (AFB) smear microscopy and the Xpert assay using culture of *Mycobacterium tuberculosis* from sputum or bronchoscopy specimens as a reference standard. A total of 132 patients were included in the final analysis. Of these, 38 had culture-confirmed PTB. The sensitivity of the Xpert assay using bronchial washing or bronchoalveolar lavage (BAL) fluid for the diagnosis of PTB was 81.6%, and specificity was 100%. The PPV and NPV were 100% and 92.1%, respectively. The sensitivity and specificity of AFB smear microscopy were respectively 13.2% and 98.8%. The Xpert assay on bronchoscopy specimens provided an accurate diagnosis of PTB in patients who had a negative AFB smear or who could not produce sputum.

Hepatotoxicity due to first-line anti-tuberculosis drugs: a five-year experience in a Taiwan medical centre

C.C. Shu, C.H. Lee, M.C. Lee, J.Y. Wang, C.J. Yu, and L.N. Lee. *The International Journal of Tuberculosis and Lung Disease* 2013; **17(7)**: 934-9.

Hepatotoxicity with first-line drugs, a major complication of anti-tuberculosis treatment, has not been studied by time-dependent analysis. Adult patients diagnosed with pulmonary tuberculosis (PTB) from 2005 to 2009 were reviewed retrospectively. Hepatotoxicity during anti-tuberculosis treatment was defined by symptomatic elevation of liver transaminases $\geq$3 times the upper limit of normal, or $\geq$5 times if asymptomatic. Risk factors for

hepatotoxicity were investigated using time-dependent Cox regression analysis. Of 926 patients identified and followed for 4122.9 person-months (pm), 111 (12.0%) developed hepatotoxicity after a median 38.0 days from start of treatment. Around 3.5% had severe hepatotoxicity. The most common symptoms were general malaise and poor appetite. The incidence rate of hepatotoxicity was 0.59, 0.69 and 3.71/100 pm for isoniazid, rifampicin (RMP) and pyrazinamide (PZA), respectively. Old age, female sex, autoimmune disease, human immunodeficiency virus infection, more days with PZA in the last 8-14 days, and fewer days with RMP in the last 15-21 days before hepatotoxicity were independent risk factors for hepatotoxicity during treatment. A significant number of adult patients on first-line treatment experience hepatotoxicity. PZA is the most common causative drug. For high-risk patients, careful adjustment of the anti-tuberculosis regimen and regular monitoring of liver transaminases are necessary.

Smoking in tuberculosis patients increases the risk of infection in their contacts

P. Godoy, J.A. Cayla, G. Carmona, N. Camps,J. Alvarez, M. Alseda, S. Minguell, A. Rodes, N. Altet, J.M. Pina, I. Barrabeig, A. Orcau, I. Parron, J. March, N. Follia, M. Sabater and A. Dominguez. *The International Journal of Tuberculosis and Lung Disease* 2013; **17(6)**: 771-6.

The objective of the study was to determine the risk of latent tuberculous infection (LTBI) among contacts of smokers with tuberculosis (TB). The study was conducted to determine the prevalence of LTBI among contacts of TB cases aged >14 years in Catalonia, Spain. A survey was carried out for each TB case and their contacts. LTBI was diagnosed using the tuberculin skin test (≥5 mm). The risk of LTBI associated with smoking was determined by multi-variate logistic regression analysis, with adjusted odds ratio (aOR) and their 95% confidence intervals (CI). The smoking prevalence among TB cases was 40.7% (439/1079). The prevalence of LTBI among their contacts was 29.7% (2281/7673). It was higher among contacts of smoking index cases (35.3%) than among those of non-smokers (25.7%). Smoking was independently associated with an increased risk of LTBI among contacts (aOR 1.5, 95%CI 1.3-1.7), and was estimated to be responsible for 12.8% of infections. Index case smoking increases the risk of LTBI and should be systematically investigated. A reduction in smoking could lower the risk of infection substantially.

Can tuberculous pleural effusions be diagnosed by pleural fluid analysis alone?

S.A. Sahn, J.T. Huggins, M.E. San Jose, J.M. Alvarez-Dobano and L. Valdes. *The International Journal of Tuberculosis and Lung Disease* 2013; **17(6)**: 787-93.

The objective of the study was to assess whether pleural fluid analysis (PFA) can confidently diagnose tuberculous pleural effusion (TPE). PFA of 548 TPEs was performed between January 1991 and December 2011. The control group consisted of patients with malignant PE (MPE), complicated parapneumonic/empyema (infectious) PE (IPE), miscellaneous PE (MisPE) and transudative PE (TrPE). The PFA of 548 histologically or culture-positive consecutive cases of TPE was compared with that of 158 consecutive cases of MPE, 113 cases of IPE, 37 cases of MisPE and 115 cases of TrPE. Statistically significant differences were noted in pleural fluid glucose, pH, cholesterol, triglycerides, adenosine deaminase (ADA), and total percentages of lymphocytes, neutrophils and macrophages when TPEs were compared to all other groups. Of the TPEs, 99.1% were exudates. Pleural fluid protein ≥ 5.0 g/dl, lymphocytes > 80% and ADA > 45 U/l were diagnostic of TPE, with a specificity of 100%, a sensitivity of 34.9% and an area under the curve of 0.975. PFA alone was diagnostic in one third of the TPE cases, with a high probability in nearly 60%.

Is a 4-month regimen adequate to cure patients with non-cavitary tuberculosis and negative cultures at two months?

P.P.J. Phillips, A.J. Nunn and N. Paton. *The International Journal of Tuberculosis and Lung Disease* 2013; **17(6)**: 807-9.

A recent trial evaluating a 4-month regimen of standard drugs in adults with non-cavitary tuberculosis (TB) and negative cultures at two months failed to demonstrate equivalence compared to the same regimen given for six months. To contribute further evidence, data from two trials conducted by the British Medical Research Council (BMRC) comparing four and six month regimens were re-analysed. The results from the BMRC trials in patients with non-cavitary TB and negative cultures at two months were consistent with those from the recent trial. However, given that there was no acquired drug resistance, the estimated 6.6% relapse rate (95%CI 4.3-10.1) across all three trials might be considered acceptable for a four-month regimen in patients with non-cavitary pulmonary TB.

Profile of Lung Cancer in Predominantly Bidi Smoking Rural Population of Northern Himachal Pradesh

P.K. Sharma and R. Bansal. *Indian J Chest Dis Allied Sci* 2013; **55**: 75-8.

Lung cancer is a leading cause of morbidity and mortality among both genders. The histopathological patterns of lung cancer in different parts of India appear to be variable. The objective was to study the profile of lung cancer in northern Himachal Pradesh. Patients of all age groups and either gender with history and complaints suggestive of lung cancer were subjected to further investigations to study the histopathological types of lung cancer over a period of 14 months. Out of 105 histopathologically confirmed patients with lung cancer (mean age 62.7±11.6 years; 96 males), 89.5% were "ever smokers" and 82.9% were "current smokers"; 92% of current smokers were bidi smokers. Most common presenting complaints were chest pain (46.7%) and cough (35.2%). Mean duration of longest presenting complaint was 64 days. The histopathological types included squamous cell carcinoma (37.1%), adenocarcinoma (36.2%), small cell carcinoma (8.6%), un-classifiable (16.2%), and other types (1.9%). Majority of the lung cancer patients in northern Himachal Pradesh were bidi smoking males from rural areas and the incidence of adenocarcinoma and squamous cell carcinoma is almost equal.

Tuberculosis cases missed in primary health care facilities: should we redefine case finding?

M.M. Claassens, E. Jacobs, E. Cyster, K. Jennings, A. James, R. Dunbar, D. Enarson, M.W. Borgdorff and N. Beyers. *The International Journal of Tuberculosis and Lung Disease* 2013; **17(5)**: 608-14.

This study was conducted in Cape Town in two primary health care facilities in a sub-district with a high prevalence of bacteriologically confirmed pulmonary tuberculosis (TB). The objective of the study was to determine the proportion of adults with respiratory symptoms who attend health care facilities but are not examined for nor diagnosed with TB in facilities where routine TB diagnosis depends on passive case finding. A total of 423 adults with respiratory symptoms exiting primary health care services were consecutively enrolled during April-July 2011. Twenty-one (5%) participants were diagnosed with culture-positive TB. None had sought care at the facility for their respiratory symptoms, none were asked about respiratory symptoms during their visit and none were asked to produce a sputum sample. Nine cases had attended the facility for reasons regarding their own health, while 12 cases were accompanying someone else attending the facility, or for another reason. Patients with infectious TB attend primary health care facilities, but are not recognised and diagnosed as cases. Health care staff should search actively within facilities for cases who attend the health care services to ensure that cases are not missed. Intensified case finding should start within the facility, and should not be limited to patients who report respiratory symptoms or who are human immunodeficiency virus positive.

Sputum collection and disposal among pulmonary tuberculosis patients in coastal South India

T. Rekha, P. Singh, B. Unnikrishnan, P. Prasanna Mithra, N. Kumar, K.D.V. Prasad, V. Raina, M. Kumar Papanna and V. Kulkarni. *The International Journal of Tuberculosis and Lung Disease* 2013; **17(5)**: 621-3.

Safe sputum disposal practices minimise the spread of pulmonary tuberculosis (PTB). The objective was to study the perceptions and practices of sputum disposal among PTB patients. This study was conducted among 206 diagnosed sputum-positive TB patients registered in selected DOTS centres in Mangalore. Safe sputum disposal practice was followed by 50% of the subjects: it was higher among females (62%), patients of middle socio-economic status (75.5%) and those with a family history of TB (70%). Furthermore, 75% patients believed that TB was caused by several factors. High proportions of subjects were unaware about the causes of TB and did not practise safe disposal of sputum.

Tuberculosis is associated with increased lung cancer mortality

C.C. Leung, L. Hui, R.S.Y. Lee, T.H. Lam, W.W. Yew, D.S.C. Hui, R.C.Y. Chan, T.Y.W. Mok, W.S. Law, K.C. Chang, E.C.C. Leung and C.M. Tam. *The International Journal of Tuberculosis and Lung Disease* 2013; **17(5)**: 687-92.

The study subjects were elderly persons living in the community in Hong Kong. The objective of the study was to examine the association between tuberculosis (TB) and lung cancer. Elderly clients enrolled in a health programme from 2000 to 2003 were retrospectively cross-matched with the territory-wide TB notification registry for TB before enrolment. The cohort was followed up prospectively through linkage with the territory-wide death registry for cause of death until 31 December 2011. All subjects with suspected malignancy or recent weight loss (≥5%) at enrolment and deaths within the first two years of follow-up were excluded. Of the 61 239 subjects included, 516 had TB before enrolment. After 490 58 person-years of follow-up, respectively 1344, 910 and 2003 deaths were caused by lung cancer, other tobacco-related malignancies and non-tobacco-related malignancies. TB before enrolment was associated with death due to lung cancer (Mantel-Haenszel weighted relative risk 2.61, 95%CI 1.82-3.74, $P < 0.001$) but not other malignancies after stratification by sex. TB remained an independent predictor of lung cancer death (adjusted hazard ratio 2.01, 95%CI 1.40-2.90; $P < 0.001$), after adjustment for multiple potential confounders. TB was independently associated with subsequent mortality due to lung cancer. This finding calls for intensification of tobacco control and better targeting of lung cancer screening in high TB burden areas.

LIST OF REVIWERS

Agarwal, Nishi, *Delhi*
Agarwal, Upasna, *Delhi*
Ahluwalia, Gautam, *Ludhiana*
Alladi Mohan, *Tirupati*
Anand Kuljeet, *Delhi*
Arora, Raksha, *Noida*
Arora, V.K, *Noida*
Banavaliker, J.N. *Delhi*
Bedi, R.S. *Patiala,*
Beena, Chennai
Behera, D. *Chandigarh*
Chadha, V.K. *Bangalore*
Chopra, K.K. *Delhi*
Chopra , Vishal,. *Ludhiana*
Dewan, R.K. *Delhi*
Gupta, Dheeraj, *Chandigarh*
Gupta, Gajendra
Gupta, K.B. *Rohtak*
Gupta, Kumud, *Delhi*
Hanif, M. *Delhi*
Jagdish Chander, *Chandigarh*
Jaikishan, *Patiala*
Jawahar, M.S. *Chennai*
Joshi, J.M. *Mumbai*
Kannan, A.T., *Delhi*
Kumar, Prahlad, *Bangalore*
Mohanty, K.C. *Mumbai*
Mohapatra, Prasanta, *Chandigarh*
Nandini Sharma, Delhi
Narang, P. *Wardha*
Padmapriyadarsini, *Chennai*
Panda, Naresh, *Chandigarh*
Poonam Goyal
Puri, M.M, *Delhi*
Purty, Anil, *Puducherry*
Radhakrishna, S, *Hyderabad*
Raghunath, D. *Bangalore*
Rajendra Prasad, *Lucknow*
Rajkumar, *Delhi*
Sarat P Chandra, *Delhi*
Sarin, Rohit, *Delhi*
Sarman Singh, *Delhi*
Sehgal, Rakesh, *Chandigarh*
Selvakumar, N. *Chennai*
Shah, Ashok, *Delhi*
Sharma, J.B. *Delhi*
Sharma, S.K. *Delhi*
Singla, Rupak, *Delhi*,
Singla, Neeta, *Delhi*
Singhal, Surender, *Chandigarh*
Sethi, Sunil, *Chandigarh*
Solanki, *Ahmedabad*
Surya Kant, *Lucknow*
Subhakar Kandi, *Hyderabad*
Surinder Kumar, *Delhi*
Swaminathan, Soumya, *Chennai*
Tripathy, S.N. *Cuttack*
Tripathy, S.P. *Agra*
Tripathi Reva, *Delhi*
Verma, Ajay, *Delhi*
Vinay Kamal, *Delhi*
Varinder Singh, *Delhi*

(Names are in alphabetical order)