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Editorial

BENEFITS OF EARLY ANTI-RETROVIRAL THERAPY IN PATIENTS WITH HIV-TB CO-INFECTION

[*Indian J Tuberc 2009; 56:113-116*]

Globally, 33 million people were estimated to be living with HIV/AIDS in 2007[1]. The number of HIV-positive TB cases and deaths were estimated at 1.39 million cases (15% of all incident cases) and 0.48 million deaths, which was 24% of the estimated two million HIV deaths in 2007[2]. In India, the 2006 estimates suggested that national adult HIV prevalence in India was approximately 0.36 per cent, amounting to 2.34 million (ranging between 2 and 3.1 million) people living with HIV and AIDS[3]. Even going by the conventional figure of 40% of the Indian population infected with *Mycobacterium tuberculosis*, it is estimated that not less than one million persons with HIV are co-infected with TB. Considering the fact that the lifetime risk of developing TB disease is between 50-60%, India is likely to have not less than one lakh patients, needing treatment for both HIV and TB simultaneously at any given point of time.

Free Anti-Retroviral Therapy (ART) was introduced in India in April, 2004, as a component of care, support and treatment, in National AIDS Control Programme (NACP). The concept of managing HIV disease in India till that time was to treat the opportunistic infections, as and when these were identified. The most common opportunistic infection, tuberculosis, was being treated by the physicians with their own selective drug schedules with their own preferential rhythm and duration of administration. Prevailing stigma and discrimination, tagged with HIV/AIDS and the absence of a robust referral and linkage system between Revised National Control Programme (RNTCP) and NACP during those times denied a large number of patients to the benefits of effective anti TB treatment protocol, available through Directly Observed Treatment Strategy even those times.

The first ever published work[4] on the effectiveness of RNTCP treatment schedule was carried out at Government Hospital of Thoracic Medicine, Tambaram, the largest TB and HIV care centre in India, by Tuberculosis Research Institute through a collaborative initiative in 1999-2000. This prospective observational feasibility study of 71 patients with HIV and tuberculosis, who were treated with category I regimen showed a favourable response in 72% of patients[5]. Even though the early bacteriological response to RNTCP regimen was satisfactory, the overall outcome was adversely affected by the high mortality (37% during treatment and 24 months of follow-up) and high recurrence rate 37%. This was essentially due to the deterioration of immune system during anti-tuberculosis treatment, with the base level mean CD4% seen falling down from 12.6 (5.9) to 8.9 (4.9) at the end of treatment ($p < 0.001$). This finding highlighted the need for the initiation of ART in addition to anti-tuberculosis treatment to improve the immune system and thereby the long term treatment outcome.

Initiation of ART reduces risk of further HIV-related morbidity and mortality. The impact of ART on HIV-infected tuberculosis TB patients was amply demonstrated in South Africa and South East Asia. HAART reduced the incidence of HIV-1-associated tuberculosis by more than 80% (95% CI 62–91) in an area endemic with tuberculosis and HIV-1 in a study conducted at Cape Town, South Africa[6]. In an observational study in Taiwan, ART was found to decrease the incidence rate of new HIV-TB co-infection cases and increased the survival rate of HIV-TB co-infection cases. The survival rate of HIV-TB co-infection cases was 62.16% during the period 1993–1996 (pre-free HAART era) and increased to 86.60% during the period 1998–2006, a post-free ART era ($P < 0.0001$)[7]. ART had brought a substantial reduction in deaths during TB treatment for HIV infected TB patients in Thailand as well. Of the patients with known outcomes, death during TB treatment occurred in 5 (7%) of 71 who received ART as against 94 (43%) of 219 who did not[8]. The impact of ART in public health programme in Thailand was in evidence, as patients who received ART had one sixth the risk of death of those not receiving ART[9]. The survival benefit persisted even for those with a very low CD4 count was a significant observation.

While access to anti-retroviral therapy is rapidly expanding in resource-limited settings, tuberculosis continues to be the most challenging opportunistic disease in HIV infected patients even after initiating anti-retroviral therapy. India had successfully rolled out first line ART to just over 220 thousand patients during the last five years, ever since its initiation in 2004. There is an increasing need for the co-administration of anti-tuberculosis and anti-retroviral treatment in patients with HIV-TB co-infection. However, the ground reality has been disturbingly indifferent. The unpublished observations available to Central TB Division from Trichy and Mysore districts of Tamilnadu and Karnataka respectively, suggested that only 26% of 396 HIV-TB co infected patients, eligible to get ART, actually received ART. Apart from the issues of self-imposed stigma, illiteracy, inadequate information and knowledge and the programmatic issues, the most contentious issue is the decision making process of ART medical officers on “when to start ART safely in HIV patients receiving anti-tuberculosis treatment”. ART physicians’ reluctance to initiate ART to HIV-infected TB patients remains a global issue, in spite of the existing national guidelines and international recommendations. They are really concerned about overlapping toxicity, drug-drug interactions, pill burden and immune reconstitution inflammatory syndrome (IRIS).

The underlying mechanisms in the development of TB after initiation of ART are indeed complex[10]. IRIS is one of the manifestations of ‘‘ART-associated TB’’ and this refers to the severe and overtly exaggerated inflammatory effects of TB. IRIS is characterised by worsening of systemic symptoms, transient enlargement of pre-existing lesions, onset of new lesions including lymphadenopathy and worsening of radiographic changes. The frequency of IRIS in cohort studies varied markedly between 8% and 43%[7,8] The mean interval to IRIS after ART initiation also varied widely (1 - 180 days) with most cases occurring within the first 28 days[11]. The risk of mortality associated with delays in ART initiation has to essentially outweigh the concerns for the possible IRIS and its outcome. The optimal timing of ART initiation may therefore be earlier in the course of TB treatment for patients in resource-limited settings with high prevalence of TB. Early ART was favoured, even in the settings of South Africa, with the highest reported rates of IRIS (70%) and severe drug toxicity (56%). ART can be deferred in settings, where IRIS-related mortality rate was

found to exceed 4.6%. These results support early initiation of ART in patients with AIDS, except when IRIS-related mortality rates are high[12]. In a larger cohort of 2330 patients with HIV initiated on ART in India, tuberculosis-associated immune reconstitution disease was found in 81 of them (3.5%) only. Even though the risk of developing IRIS was expected to be high, on being initiated with ART in those with low baseline CD4 cell counts, manifestations of IRIS and risk of mortality in Indian patients were found to be self-limiting, favouring early initiation of ART in patients with HIV-TB co-infection[13].

ART reduces the incidence of TB in treated cohorts even in high TB prevalence countries. Two hundred and sixty-two patients (5.1%) of 5099 patients with as many with 88% patients had base level CD4 count less than 200/mm^3, followed-up for one to four years were found to have Post ART TB with 100-person year risk of 2.83 in the largest Indian cohort[14]. In a Cape Town cohort of 346 patients receiving HAART between 1996 and 2005, TB incidence rate was observed to be 3.5/ 100 person-years in the first year and significantly decreased during follow-up, reaching 1.01/100 person-years in the fifth year (P = 0.002 for trend) [15]. In spite of beneficial effect of ART, incidence of post-ART TB continues to be on the higher side than those among HIV-negative individuals. Current data suggest that ART can achieve suboptimal restoration of MTB-specific immune responses only. Hence, the contribution of ART is limited to patients alone and not to the community, as a significant number patients receiving ART live much longer and yet would maintain a chronically heightened risk of TB[16].

HIV associated TB is a major public health problem. Tuberculosis services are an important entry point for identifying ART eligible patients. Given that dually infected patients identified through tuberculosis services contributed to 10% of the HIV-infected adult population with a CD4 cell count below 350 cells/mm^3 in the 18 sub-Saharan African countries[17]. The burden of HIV among tuberculosis patients varies widely in India, from 1% in Koch Bihar, West Bengal, to 13.8% in Guntur, Andhra Pradesh[18]. Programme efforts to implement comprehensive TB-HIV services should be targeted to areas with the highest HIV burden districts through the Intensified TB Screening mechanism. A simple diagnostic tool, evaluating the common signs and symptoms like oral thrush, diarrhoea, itching in 25-45 year old adults indulging in high risk behaviour could be used to screen patients for HIV at DOTS centres to Integrated Counselling and Testing Centres for early detection of HIV-TB co-infection[19]. Simultaneously, the number of HIV infected persons to have been screened for TB has to be strengthened through Intensified TB Screening. The existing policy of NACP to screen all the patients with HIV sero-positivity for identifying CD4 counts augurs well for identifying the eligible patients with HIV-TB co-infection for early ART initiation. The revised ART guidelines are strongly in favour of initiation ART early in all TB patients with HIV, having CD4 count less than 350 cells/mm^3 after two weeks of anti-tuberculosis patients. Patients with extra-pulmonary TB, being a WHO clinical stage IV disease, have the additional advantage of getting ART initiated irrespective of CD4 cell count.

Having realised the need for early identification of HIV-TB co-infection and for early initiation of ART in them, it is the moment to prioritise the course of action. Dissemination of information, knowledge and national guidelines among the concerned health care workers through a well planned

training programme is the need of the hour. All the health care professionals connected with RNTCP and NACP should join together and strengthen the collaborative activities, referrals, linkages and monitoring mechanism to translate the light of wisdom into fruit of benefit for the needy.

Dr. S. Rajasekaran
NACO National Consultant (ART Quality Management)
Chennai

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Original article

ASSOCIATION OF 22 CYTOKINE GENE POLYMORPHISMS WITH TUBERCULOSIS IN MACEDONIANS

Dejan Trajkov[1], Mirjana Trajchevska[2], Todor Arsov[1], Aleksandar Petlichkovski[1], Ana Strezova[1], Olivija Efinska-Mladenovska[1], Aleksandar Sandevski[1] and Mirko Spiroski[1]

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Summary

Objective: To examine the possible role of 22 cytokine gene polymorphisms in host susceptibility to or protection against tuberculosis (TB) in Macedonians.

Method: 301 healthy unrelated individuals and 75 patients with pulmonary TB were studied. Cytokine genotyping was performed by PCR with sequence-specific priming (PCR-SSP) (Heidelberg kit).

Results: TNF-Ü-238/G, IL-1R psti1970/C, IL-1â +3962/T:T, IL-4 -1098/ T:T, IFNã utr5644/A:A, IL-10 -1082/G:G, IL-4 -590/C:C, IL-10/ATC, IL-4/TCT, IL-4/TCC, IL-10/ATC:GCC, IL-4/TCT:TTT, IL-4/TCC:TTC, IL-10/GCC:GCC and IL-4/TCC:TCC were positively associated with TB, while protective association was identified for IL-4 -1098/G, IL-1â +3962/C, IFNã utr5644/T, IL-1â +3962/C:T, IL-4 -1098/G:T, IL-4 -590/C:T, IFNã utr5644/A:T, IL-4/GCC, IL-4/TTC and IL-4/GCC:TTC.

Conclusion: These results suggest that some cytokine polymorphisms are significantly associated and affect host susceptibility/resistance to TB in Macedonians. **[*Indian J Tuberc 2009; 56:117-131*]**

***Key words*:** TB, Cytokine polymorphism, Macedonians.

INTRODUCTION

Tuberculosis (TB) represents itself as a major health problem globally. [1] The incidence of disease has increased in developed countries[2] and, according to World Health Organization's estimations, with four millions deaths annually, TB is one of the leading death causing diseases.[3, 4] Although one third of the world's population is infected with *M. tuberculosis*, the fact that only 10% of those who are infected develop tuberculosis points out the role of genetic factors in the pathogenesis of the disease.[5] The best evidence that genetic factors are very important in susceptibility and resistance to tuberculosis comes from the familial clustering, familial differences in incidence, and twin studies showing that concordance among identical twins was 65-85% and 25-35% for non-identical ones.[6-9]

Each stage of the host response to *M. tuberculosis* is under genetic control, including the initial encounter with mycobacteria by macrophages, epithelial cells and dendritic cells in the lung, induction of the inductive T cell response, and killing by activated macrophages within granulomas.[10, 11] Although environmental factors are important determinants of progression to disease, there is a genetic component underlying susceptibility to tuberculosis (TB), the basis of which may vary in different populations.[12] Crucial factors in resistance to *M. tuberculosis* lie in macrophage activation and Th1 type lymphocyte response.[13] Cytokines that are produced at the site of infection and influence activation of these cells, highly participate in the pathogenesis of TB.[14] Manifestation of clinical TB depends on balance between T helper 1 (Th1) cytokines associated with resistance to infection, and Th2 cytokines associated with progressive disease.[15] Factors that influence the nature of

1. Institute of Immunobiology and Human Genetics, Faculty of Medicine, University "St. Kiril and Metodij", Skopje, Republic of Macedonia 2. Institute for Tuberculosis, Skopje, Republic of Macedonia

Correspondence: Mirko Spiroski, MD, PhD., Institute of Immunobiology and Human Genetics, Faculty of Medicine, University "Ss. Kiril and Metodij", 1109 Skopje, PO Box 60, Republic of Macedonia; Tel.: +389-2-3110556; Fax: +389-2-3110558; URL: http://www.immunology.edu.mk; E-mail: mspiroski@yahoo.com

cytokine response, such as polymorphisms of cytokine genes, will lead to modification of host immunological response. Although mechanisms of altered gene expression associated with polymorphisms are still poorly understood, there is more and more evidence that sequence changes in cytokine genes may result in altered transcription factor recognition sites, affecting transcriptional activation and influence production of the corresponding peptide solely or due to linkage with another marker directly affecting gene expression.[16,18]

We have previously published data for the cytokine polymorphisms in healthy Macedonian population.[19] The aim of this study was to investigate the existence of possible associations between 22 cytokine genes polymorphisms and TB in Macedonians.

MATERIAL AND METHODS

Groups

The total studied sample consisted of 376 examinees, divided into two different groups as follows: healthy individuals, and patients with tuberculosis.

Healthy individuals. There were 301 unrelated individuals, born in different parts of Macedonia. They were age and sex non-matched healthy individuals who attended the Institute of Immunobiology and Human Genetics for DNA donation between May 1, 2001 and April 25, 2002 and agreed to take part in this study as a control group. Individuals with family history of tuberculosis were excluded from the investigation.

Tuberculosis. There were 75 patients with tuberculosis fulfilling the criteria of diagnostic algorithm for TB diagnosis recommended by WHO.[20] They were 20-59 years old consecutive patients who attended the Institute for Tuberculosis, Skopje, Republic of Macedonia for treatment between January 10, 2003 and April 25, 2004.

All individuals were of Macedonian origin and nationality, and residents of different regions of the Republic of Macedonia. Each individual was interviewed on a one-to-one basis, his/her genealogy was recorded for the last three generations, and a signed consent was obtained. Admixture, if any, was recorded for each individual. Individuals with only one Macedonian parent was excluded from the study.

All the patients and healthy individuals included in this study signed a written consent to participate in the study which was approved by the Committee of the Ministry of Education and Science from Republic of Macedonia (No13-874/3-05), and Ethical Committee of the Medical Faculty in Skopje.

Genomic DNA Isolation and Storage

DNA was isolated from peripheral blood leukocytes by phenol-chloroform extraction method or with BioRobot EZ1 workstation (QIAGEN).[21] The quality and quantity of DNA was analyzed by GeneQuant (Pharmacia Biotech, Uppsala, Sweden). Isolated DNA samples were stored in the Macedonian Human DNA Bank.[22]

Typing Methods

Cytokine genotyping was performed by PCR-SSP (Heidelberg kit). Fourteen cytokine genes with 22 single nucleotide polymorphisms (SNP) were typed: *IL-1Ü-889, IL-1â -511, IL-1â +3962, IL-1R psti1970, IL-1RA mspa11100, IL-4RÜ+1902, IL-12 -1188, IFNã utr5644, TGF-â1 cdn10, TGF-â1 cdn25, TNF-Ü-308, TNF-Ü-238, IL-2 -330, IL-2 +166, IL-4 -1098, IL-4 -590, IL-4 -33, IL-6 -174, IL-6 565, IL-10 -1082, IL-10 -819,* and *IL-10 -592*. Briefly, PCR-SSP typing Heidelberg kit consists of 48 PCR primer mixes aliquotted in 96 well PCR trays (two typings per tray). Master mix, which was supplied along with the reagents and consisted of $MgCL_2$, buffer, dNTP's, and glycerol was mixed with 1.2 - 3.0 µg DNA and 20 U Taq polymerase and dispensed in the 48 wells.[23] Agarose gel electrophoresis on a 2% gel revealed a positive or

negative signal for specific amplification in each well. Subsequently, the results were analyzed according to the interpretation scheme provided with the kit.[24]

Statistical Methods

The population genetics analysis package, PyPop, developed by the Biostatistics Core for the Workshop,[25-27] was used for analysis of the cytokine data in this study. Allele frequencies and expected Hardy Weinberg proportions (HWP) for each SNP were determined.[28] The exact test for genotype frequency deviation from HWP was calculated using the Arlequin implementation accessed via PyPop.[29] Those SNPs that did not fit HWP were evaluated to determine whether there was an excess of homozygotes or heterozygotes, or if any particular genotype frequencies were significantly different from the expected frequencies. Comparisons of different genotypes for two groups were tested by the χ^2 test. Crude odds ratios (OR), as estimates of the relative risk, were calculated within 95% CI.

RESULTS

Cytokine Alleles

In Table 1, frequencies of polymorphic cytokine alleles, Fisher exact p-value, Odds ratio and Wald's 95% confidence interval in TB patients and healthy Macedonians are shown.

For the members of IL-1 gene cluster, we found positive association for *IL-1R psti1970/T* allele (p=0.006, **OR=1.804**, Wald's 95% CI between 1.183-2.750), while *IL-1â +3962/C* allele showed negative (protective) association (p=0.0002, **OR=0.501**, Wald's 95% CI between 0.345-0.727) for TB. In the IL-12/IFNã axis, protective association was obtained for the *IFNã utr5644/T* allele (p=0.004, **OR=0.567**, Wald's 95% CI between 0.384-0.838). Results showed that people with *TNF-Ü-238 /G* allele have **5.141** fold risk to develop TB (p<0.0001, Wald's 95% CI between 3.014-8.769) in comparison to others with *TNF-Ü-238 /A* allele. Analysis of the IL-4 polymorphisms showed that *IL4 -1098/G* allele was associated with TB (p=0.0001, **OR=0.408**, Wald's 95% CI between 0.253-0.657) (Table 1).

Cytokine genotypes

Table 2 contains summarized results for different cytokine genotypes found in our study.

Analysis of all possible IL-1 gene cluster genotypes showed that only heterozygous genotype of *IL-1â +3962* polymorphism was protectively associated with TB (p=0.0002, **OR=0.241**, Wald's 95% CI between 0.107-0.546), while homozygous */T:T* genotype of the same polymorphism showed susceptible effect (p<0.001, **OR=4.481**, Wald's 95% CI between 2.497-8.041). In the IL-12/IFNã axis two genotypes of *IFNãutr5644* polymorphism showed association with TB. While */A:A* genotype was positively associated (p=0.0004, **OR=2.643**, Wald's 95% CI between 1.514-4.615), */A:T* genotype showed negative association with TB (p=0.017, **OR=0.492**, Wald's 95% CI between 0.274-0.885). Two observed genotypes of IL-4 polymorphisms were positively associated with TB (*IL-4 -1098/T:T*, p<0.0001, **OR=3.564**, Wald's 95% CI between 2.066-6.150; and *IL-4 -590/C:C*, p=0.018, **OR=1.856**, Wald's 95% CI between 1.108-3.108), while heterozygous genotypes of the same IL-4 polymorphisms showed protective association: *IL-4 -1098/G:T* (p<0.0001, **OR=0.285**, Wald's 95% CI between 0.165-0.491) and *IL-4 -590/C:T* genotype (p=0.006, **OR=0.489**, Wald's 95% CI between 0.292-0.817). We found that patients with *IL-10 -1082/G:G* genotype have 2.5 higher risk to develop TB (p<0.022, **OR=2.552**, Wald's 95% CI between 1.117-5.831). Homozygous genotypes *IL-1R psti1970/T:T, TNF-Ü-238/A:A* and *IL-4 -1098/G:G* were present only in patients with TB. Neither healthy Macedonian population nor patients with TB have *TGF-â1 cdn25/C:C* genotype (Table 2).

Cytokine Haplotypes

For several genes with multiple SNPs per gene (*TGF-â1, TNF-Ü, IL-2, IL-4, IL-6, IL-10*)

Table 1: Cytokine allele frequency, Fisher exact p-value, Odds ratio and Wald's 95% confidence interval in TB patients and healthy Macedonians

Cytokine Polymorphism	Allele	TB (n=75)		Control (n=301)		Fisher exact p-value	Odds ratio	Wald' 95% CI
		N	F	N	F			
IL-1ά -889	C	120	0.811	482	0.814	0.925	0.978	0.617-1.550
	T	28	0.189	110	0.186			
IL-1 -511	C	106	0.707	404	0.671	0.404	1.181	0.799-1.745
	T	44	0.293	198	0.329			
IL-1 +3962	C	85	0.574	439	0.729	0.0002	0.501	0.345-0.727
	T	63	0.426	163	0.270			
IL-1R psti1970	C	117	0.780	399	0.662	0.006	1.804	1.183-2.750
	T	33	0.220	203	0.337			
IL-1RA mspa11100	T	103	0.696	420	0.698	0.967	0.992	0.671-1.467
	C	45	0.304	182	0.302			
IL-4Rά +1902	A	127	0.847	502	0.834	0.705	1.100	0.672-1.801
	G	23	0.153	100	0.166			
IL-12 -1188	A	113	0.753	433	0.744	0.815	1.051	0.694-1.592
	C	37	0.247	149	0.256			
IFN utr5644	T	51	0.381	259	0.520	0.004	0.567	0.384-0.838
	A	83	0.619	239	0.480			
TGF- 1 cdn10	T	71	0.473	282	0.502	0.536	0.892	0.622-1.280
	C	79	0.527	280	0.498			
TGF- 1 cdn25	G	141	0.940	532	0.947	0.752	0.884	0.410-1.904
	C	9	0.060	30	0.053			
TNF-ά -308	A	15	0.100	74	0.123	0.437	0.793	0.441-1.425
	G	135	0.900	528	0.877			
TNF-ά -238	A	35	0.033	27	0.045	<0.0001	5.141	3.014-8.769
	G	145	0.967	575	0.955			
IL-2 -330	G	58	0.387	191	0.332	0.216	1.264	0.872-1.833
	T	92	0.613	383	0.667			
IL-2 +166	G	114	0.760	422	0.735	0.537	1.141	0.751-1.733
	T	36	0.240	152	0.264			
IL-4 -1098	G	23	0.153	176	0.308	0.0001	0.408	0.253-0.657
	T	127	0.847	396	0.692			
IL-4 -590	C	108	0.720	377	0.659	0.157	1.330	0.895-1.977
	T	42	0.280	195	0.341			
IL-4 -33	C	118	0.787	479	0.837	0.144	0.716	0.457-1.122
	T	32	0.213	93	0.163			
IL-6 -174	C	47	0.313	182	0.302	0.793	1.053	0.716-1.550
	G	103	0.687	420	0.698			
IL-6 nt565	A	47	0.313	173	0.287	0.532	1.132	0.768-1.667
	G	103	0.687	429	0.713			
IL-10 -1082	A	82	0.547	352	0.589	0.352	0.843	0.588-1.208
	G	68	0.453	246	0.411			
IL-10 -819	C	105	0.700	435	0.727	0.503	0.874	0.590-1.295
	T	45	0.300	163	0.272			
IL-10 -592	A	41	0.273	173	0.289	0.699	0.924	0.619-1.379
	C	109	0.727	425	0.710			

N= absolute number; F=frequency; CI=Confidence Interval; *, statistically significant.

Table 2: Cytokine genotype frequency, Fisher exact p-value, Odds ratio and Wald's 95% confidence interval in TB patients and healthy Macedonians

Polymorphism	Geno -type	TB (n=75)		Controls (n=301)		Fisher exact p-value	Odds ratio	Wald' 95% CI
		N	F	N	F			
IL-1ά -889	C:C	50	0.676	204	0.689	0.823	0.940	0.545-1.621
	C:T	20	0.270	74	0.250	0.720	1.111	0.624-1.978
	T:T	4	0.054	18	0.061	0.826	0.883	0.290-2.691
IL-1 -511	C:C	36	0.480	143	0.475	0.939	1.020	0.615-1.692
	C:T	34	0.453	118	0.392	0.333	1.286	0.772-2.142
	T:T	5	0.067	40	0.133	0.114	0.466	0.177-1.225
IL-1 +3962	C:C	39	0.527	174	0.578	0.427	0.813	0.488-1.355
	C:T	7	0.095	91	0.302	0.0002	0.241	0.107-0.546
	T:T	28	0.378	36	0.120	<0.0001	4.481	2.497-8.041
IL-1R psti1970	C:C	42	0.560	133	0.442	0.067	1.608	0.966-2.676
	C:T	33	0.440	133	0.442	0.977	0.993	0.596-1.652
	T:T	0	/	35	0.116	§	§	§
IL-1RA mspa11100	C:C	7	0.095	30	0.100	0.897	0.944	0.397-2.242
	C:T	31	0.419	122	0.405	0.831	1.058	0.631-1.772
	T:T	36	0.486	149	0.495	0.895	0.966	0.581-1.608
IL-4Rά +1902	A:A	55	0.733	212	0.704	0.620	1.155	0.654-2.038
	A:G	17	0.227	78	0.259	0.563	0.838	0.460-1.525
	G:G	3	0.040	11	0.037	0.888	1.099	0.299-4.041
IL-12 -1188	A:A	42	0.560	160	0.550	0.874	1.042	0.625-1.737
	A:C	29	0.387	113	0.388	0.979	0.993	0.590-1.672
	C:C	4	0.053	18	0.062	0.782	0.855	0.280-2.604
IFN utr5644	A:A	32	0.478	64	0.257	0.0004	2.643	1.514-4.615
	A:T	19	0.283	111	0.446	0.017	0.492	0.274-0.885
	T:T	16	0.239	74	0.297	0.347	0.742	0.398-1.385
TGF- 1 cdn10	C:C	13	0.173	65	0.231	0.281	0.697	0.361-1.347
	C:T	45	0.600	150	0.534	0.306	1.310	0.780-2.199
	T:T	17	0.227	66	0.235	0.881	0.955	0.520-1.752
TGF- 1 cdn25	C:G	9	0.120	30	0.107	0.744	1.141	0.516-2.521
	G:G	66	0.880	251	0.893	0.744	0.877	0.397-1.937
	C:C	0	/	0	/	§	§	§
TNF-ά -308	A:G	11	0.147	66	0.219	0.163	0.612	0.305-1.227
	G:G	62	0.827	231	0.768	0.269	1.445	0.751-2.782
	A:A	2	0.026	4	0.013	0.408	2.034	0.366-11.322
TNF-ά -238	A:G	5	0.067	23	0.076	0.774	0.863	0.317-2.352
	G:G	70	0.933	276	0.917	0.639	1.268	0.469-3.431
	A:A	0	/	2	0.007	§	§	§
IL-2 -330	G:G	9	0.120	27	0.094	0.504	1.313	0.589-2.926
	G:T	40	0.533	137	0.477	0.388	1.251	0.752-2.083
	T:T	26	0.347	123	0.429	0.199	0.708	0.417-1.202
IL-2 +166	G:G	43	0.573	162	0.565	0.890	1.037	0.620-1.733
	G:T	28	0.373	98	0.341	0.606	1.149	0.678-1.948
	T:T	4	0.054	27	0.094	0.262	0.543	0.184-1.601

Table contd. on next page .....

Polymorphism	Geno -type	TB (n=75)		Controls (n=301)		Fisher exact p-value	Odds ratio	Wald' 95% CI
		N	F	N	F			
IL-4 -1098	**G:T**	23	0.307	174	0.608	<0.0001	0.285	0.165-0.491
	T:T	52	0.693	111	0.388	<0.0001	3.564	2.066-6.150
	G:G	0	/	1	0.004	§	§	§
IL-4 -590	**C:C**	36	0.480	95	0.332	0.018	1.856	1.108-3.108
	C:T	36	0.480	187	0.654	0.006	0.489	0.292-0.817
	T:T	3	0.040	4	0.014	0.146	2.938	0.643-13.420
IL-4 -33	**C:C**	52	0.693	209	0.731	0.519	0.833	0.478-1.453
	C:T	14	0.187	61	0.213	0.613	0.847	0.444-1.615
	T:T	9	0.120	16	0.056	0.052	2.301	0.974-5.437
IL-6 -174	**C:C**	8	0.107	25	0.083	0.518	1.318	0.569-3.053
	C:G	31	0.413	132	0.439	0.694	0.902	0.540-1.507
	G:G	36	0.480	144	0.478	0.980	1.006	0.607-1.670
IL-6 nt565	**A:A**	8	0.107	25	0.083	0.518	1.318	0.569-3.053
	A:G	31	0.413	123	0.409	0.941	1.020	0.610-1.705
	G:G	36	0.480	153	0.508	0.661	0.893	0.538-1.481
IL-10 -1082	**A:A**	17	0.227	70	0.234	0.891	0.959	0.525-1.753
	A:G	48	0.640	212	0.709	0.246	0.730	0.428-1.244
	G:G	10	0.133	17	0.057	0.022	2.552	1.117-5.831
IL-10 -819	**C:C**	35	0.467	155	0.518	0.423	0.813	0.490-1.350
	C:T	35	0.467	125	0.418	0.447	1.218	0.733-2.025
	T:T	5	0.066	19	0.064	0.921	1.053	0.380-2.917
IL-10 -592	**A:A**	5	0.067	28	0.094	0461	0.691	0.258-1.855
	A:C	31	0.413	117	0.391	0.727	1.096	0.655-1.834
	C:C	39	0.520	154	0.515	0.939	1.020	0.615-1.693

N= absolute number; F=frequency; CI=Confidence Interval; § cannot be calculated because expected <5, $\chi 2$ test; *, statistically significant.

using the Heidelberg PCR-SSP kit we were able to detect true haplotypes. Cytokine haplotypes frequency in the TB patients and healthy Macedonians, together with the Fisher exact p-value, Odds ratio and Wald's 95% confidence interval are shown in Table 3.

Significant association with TB was observed in four IL-4 haplotypes and one IL-10 haplotype. Positive association was shown (according to the level of susceptibility) for *IL-10/ATC* (p=0.004), odds ratio **8.164** (1.481-45.01); *IL-4/TCT* (p=0.0004), odds ratio **6.951** (2.007-24.07) and *IL-4/TCC* (p=0.0001), odds ratio **1.984** (1.380-2.854). Negative (protective) association was found only for *IL-4/GCC* haplotype (p=0.001), odds ratio **0.454** (0.281-0.734). Haplotypes *TGF-â1/TC* and *TNF-ÜAA* were present only in healthy Macedonian population, while only TB patients had *IL-4/GCT, IL-4/GTC, IL-4/GTT, IL-6/CG, IL-6/GA* and *IL-10/ACA* haplotypes (Table 3).

Cytokine Diplotypes (Haplotype Zygosity)

Cytokine diplotypes (or haplotype zygozity) are combinations of haplotypes from both parents. Table 4 comprises results from cytokine diplotypes analysis.

Obtained results reveal that *IL-4/TCT:TTT* (p=0.009, **OR=5.036**, Wald's 95% CI between 1.318-19.24), *IL4/TCC:TTC* (p=0.006, **OR=4.103**, Wald's 95% CI between 1.392-12.09)

Table 3: Haplotype frequency of cytokine polymorphism, Fisher exact p-value, Odds ratio and Wald's 95% confidence interval in TB patients and healthy Macedonians

Polymorphism	Haplotype	TB (n=75)		Control (n=301)		Fisher exact p-value	Odds ratio	Wald' 95% CI
		N	F	N	F			
TGF-β1	CC	8	0.053	30	0.053	0.998	0.999	0.448-2.227
	CG	63	0.420	250	0.445	0.586	0.904	0.628-1.301
	TG	78	0.520	282	0.502	0.692	1.076	0.750-1.543
	TC	1	0.007	/	/	§	§	§
TNF-ά	AG	14	0.093	74	0.123	0.313	0.735	0.403-1.340
	GA	4	0.027	26	0.043	0.355	0.607	0.209-1.766
	GG	131	0.873	502	0.834	0.236	1.374	0.811-2.326
	AA	1	0.007	/	/	§	§	§
IL-2	GG	56	0.373	178	0.310	0.140	1.325	0.911-1.929
	GT	2	0.014	14	0.024	0.412	0.541	0.122-2.405
	TG	57	0.380	244	0.425	0.318	0.829	0.573-1.199
	TT	35	0.233	138	0.240	0.856	0.962	0.629-1.469
IL-4	GCC	23	0.153	163	0.285	0.001	0.454	0.281-0.734
	GCT	/	/	8	0.014	§	§	§
	GTC	/	/	4	0.007	§	§	§
	GTT	/	/	1	0.002	§	§	§
	TCC	78	0.520	202	0.353	0.0001	1.984	1.380-2.854
	TCT	7	0.047	4	0.007	0.0004	6.951	2.007-24.07
	TTC	17	0.113	110	0.192	0.024	0.537	0.311-0.927
	TTT	25	0.167	80	0.140	0.407	1.23	0.753-2.008
IL-6	CA	47	0.313	172	0.286	0.505	1.141	0.774-1.681
	CG	/	/	9	0.150	§	§	§
	GG	103	0.687	420	0.698	0.793	0.950	0.645-1.398
	GA	/	/	1	0.002	§	§	§
IL-10	ACA	/	/	12	0.020	§	§	§
	ACC	37	0.247	177	0.296	0.232	0.779	0.517-1.174
	ATA	41	0.273	161	0.269	0.919	1.021	0.683-1.526
	ATC	4	0.027	2	0.003	0.004	8.164	1.481-45.01
	GCC	68	0.453	246	0.411	0.352	1.187	0.828-1.701

N= absolute number; F=frequency; CI=Confidence Interval; § cannot be calculated because expected <5, $\chi 2$ test;*, statistically significant.

and *IL-4/TCC:TCC* (p=0.018, OR=1.910, Wald's 95% CI between 1.112-3.282) combination of haplotypes have susceptible association, while only *IL-4/GCC:TTC* (p=0.0001, **OR=0.273**, Wald's 95% CI between 0.135-0.555) has protective association with TB. Concerning IL-10 combination of haplotypes, obtained results showed that */ATC:GCC* (p=0.004, **OR=8.366**, Wald's 95% CI between 1.502-46.58) and */GCC:GCC* (p=0.022, **OR=2.552**, Wald's 95% CI between 1.117-5.831) diplotypes have positive association with TB. We found that *TGF-â1/CG:TC, TNF-ÜGG:AA, IL-2/GT:GT* and *IL-4/TCT:TCT* combination of haplotypes were present only in healthy Macedonian population. On the other hand, *TNF-ÜGA:GA; IL-2/GT:TG, /GT:GG* and */GT:TT; IL-4/GCT:TTT, /GTC:TTC* and */GTT:TTC; IL-6/CG:GG* and */GA:GG*; and *IL-10/ACA:GCC* and */ACA:ATA* diplotypes were found only in patients with TB (Table 4).

In Table 5, we can see the summary of all susceptible and protective cytokine polymorphisms obtained in our study. We can see that majority of cytokine diplotypes were positively associated with TB, while only one showed protective association. Two cytokine alleles, five cytokine genotypes and three cytokine haplotypes

Table 4: Cytokine diplotypes (haplotype zygotes), Fisher exact p-value, Odds ratio and Wald's 95% confidence interval in TB patients and healthy Macedonians

Polymorphism	Genotype	TB (n=75)		Control (n=301)		Fisher exact p-value	Odds ratio	Wald' 95% CI
		N	F	N	F			
TGF-β1	CC:CG	4	0.053	16	0.057	0.904	0.933	0.303-2.879
	CC:TG	4	0.053	14	0.050	0.902	1.074	0.343-3.365
	CG:CG	9	0.120	49	0.174	0.257	0.646	0.302-1.383
	CG:TG	40	0.533	136	0.484	0.448	1.219	0.731-2.030
	TG:TG	17	0.227	66	0.235	0.881	0.955	0.520-1.752
	CG:TC	1	0.014	0	/	§	§	§
TNF-	AG:GG	10	0.133	66	0.219	0.097	0.548	0.267-1.125
	GA:GG	4	0.053	24	0.080	0.436	0.650	0.219-1.934
	GG:GG	58	0.773	206	0.684	0.132	1.573	0.870-2.846
	AG:AG	2	0.027	4	0.013	0.408	2.034	0.366-11.32
	GG:AA	1	0.014	0	/	§	§	§
	GA:GA	0	/	1	0.004	§	§	§
IL-2	GG:GG	9	0.120	27	0.094	0.504	1.313	0.589-2.926
	GG:TG	24	0.320	85	0.296	0.689	1.118	0.647-1.933
	GG:TT	14	0.187	38	0.133	0.233	1.504	0.767-2.950
	GT:TG	0	/	11	0.058	§	§	§
	TG:TG	10	0.133	50	0.174	0.397	0.729	0.351-1.517
	TG:TT	13	0.173	48	0.168	0.900	1.044	0.532-2.047
	TT:TT	4	0.053	25	0.087	0.337	0.590	0.199-1.752
	GT:GG	0	/	1	0.003	§	§	§
	GT:TT	0	/	2	0.007	§	§	§
	GT:GT	1	0.014	0	/	§	§	§
IL-4	GCC:GCC	0	/	1	0.003	§	§	§
	GCC:TCC	7	0.093	26	0.091	0.948	1.029	0.429-2.473
	GCC:TTC	10	0.133	103	0.360	0.0001	0.273	0.135-0.555
	GCC:TTT	6	0.080	32	0.112	0.423	0.690	0.277-1.718
	TCC:TCC	28	0.373	68	0.238	0.018	1.910	1.112-3.282
	TCC:TTC	7	0.093	7	0.025	0.006	4.103	1.392-12.09
	TCC:TTT	8	0.107	28	0.098	0.822	1.100	0.480-2.524
	TTT:TTT	3	0.040	4	0.014	0.146	2.938	0.643-13.42
	GCT:TTT	0	/	8	0.028	§	§	§
	GTC:TTC	0	/	4	0.014	§	§	§
	TCT:TTT	5	0.067	4	0.014	0.009	5.036	1.318-19.24
	GTT:TTC	0	/	1	0.003	§	§	§
	TCT:TCT	1	0.014	0	/	§	§	§
IL-6	CA:CA	8	0.107	25	0.083	0.518	1.318	0.569-3.053
	CA:GG	31	0.413	122	0.405	0.899	1.034	0.618-1.728
	CG:GG	/	/	9	0.030	§	§	§
	GG:GG	36	0.480	144	0.479	0.980	1.006	0.607-1.670
	GA:GG	/	/	1	0.003	§	§	§
IL-10	ACC:ACC	4	0.053	21	0.070	0.600	0.746	0.248-2.242
	ACC:ATA	8	0.107	21	0.070	0.292	1.581	0.671-3.724
	ACC:GCC	21	0.280	114	0.381	0.103	0.631	0.362-1.100
	ATA:ATA	5	0.067	19	0.064	0.921	1.053	0.380-2.917
	ATA:GCC	23	0.307	93	0.311	0.942	0.980	0.566-1.696
	GCC:GCC	10	0.133	17	0.057	0.022	2.552	1.117-5.831
	ACA :GCC	0	/	3	0.010	§	§	§
	ACA :ATA	0	/	9	0.030	§	§	§
	ATC :GCC	4	0.053	2	0.007	0.004	8.366	1.502-46.58

N= absolute number; F=frequency; CI=Confidence Interval; § cannot be calculated because expected <5, 2 test; *, statistically significant.

Table 5: Summary of all susceptible and protective cytokine polymorphisms for TB in Macedonians

	Susceptible			Protective		
	Polymorphism	p	Odds ratio	Polymorphism	p	Odds ratio
Cytokine Alleles	TNF-α -238/G	<0.0001	5.141	IL-4 -1098/G	0.0001	0.408
	IL-1R psti1970/C	0.006	1.804	IL-1β +3962/C	0.0002	0.501
				IFNγ utr5644/T	0.004	0.567
Cytokine Genotypes	IL-1β +3962/T:T	<0.0001	4.481	IL-1β +3962/C:T	0.0002	0.241
	IL-4 -1098/ T:T	<0.0001	3.564	IL-4 -1098/G:T	<0.0001	0.285
	IFNγ utr5644/A:A	0.0004	2.643	IL-4 -590/C:T	0.006	0.489
	IL-10 -1082/G:G	0.022	2.552	IFNγ utr5644/A:T	0.017	0.492
	IL-4 -590/C:C	0.018	1.856			
Cytokine Haplotypes	IL-10/ATC	0.004	8.164	IL-4/GCC	0.001	0.454
	IL-4/TCT	0.0004	6.951	IL-4/TTC	0.024	0.537
	IL-4/TCC	0.0001	1.984			
Cytokine Diplotypes (Haplotype Zygosity)	IL-10/ATC:GCC	0.004	8.366	IL-4/GCC:TTC	0.0001	0.273
	IL-4/TCT:TTT	0.009	5.036			
	IL-4/TCC:TTC	0.006	4.103			
	IL-10/GCC:GCC	0.022	2.552			
	IL-4/TCC:TCC	0.018	1.910			

also showed susceptible association with TB. Protective association with TB was obtained for three cytokine alleles, four cytokine genotypes and two cytokine haplotypes.

DISCUSSION

Defense against *M. tuberculosis* depends upon Th1 lymphocyte response and macrophage activation.[30] Because the crucial element in host defense is prompt production of proinflammatory cytokines, cytokines that have effect upon these cells may have influence on host susceptibility/resistance to TB. Cytokines of IL-1 complex are part of the immunological response against *M. tuberculosis* and are considered mediators of tissue destruction.[31] IL-1 gene cluster contains the genes for interleukin-1Ü (*IL-1Ü*), interleukin-1â (*IL-1â*), IL-1 receptor antagonist (*IL-1RA*), interleukin-1 receptor (*IL-1R*) and interleukin-18 (*IL-18*), and has been mapped on chromosome 2q13-14, within a region of 450 kb.[32,33] IL-1â is a proinflammatory cytokine. Main source of its production are monocytes/ macrophages, but fibroblast, B-cells, dendritic cells, endothelial cells and other cell types can also produce IL-1â. It is produced at the site of infection during tuberculosis (TB) and induces production of other cytokines from a variety of cells, thereby resulting in further macrophage activation and facilitating infiltration by other mononuclear cells.[34, 35] IL-1â also has a role in directing T-cell responses during infection and

granuloma formation.[36] While IL-1Ü and IL-1â are potent proinflammatory cytokines involved in the early recruitment of inflammatory cells to *M. tuberculosis* induced granulomas,[37,38] IL-1RA is an anti-inflammatory cytokine. All three molecules bind to the same interleukin-1 receptor (IL-1R)[39] and compete among each other. In our study we evaluated the role of two SNPs in *IL-1â* gene *(-511 C/T* and *+3962 C/T*), one polymorphism in *IL-1Ü* gene *(-889 C/T*), one polymorphism in *IL-1R* gene (*psti1970 C/T*) and one polymorphism in *IL-1RA* gene (*mspa11100 T/C*) in the pathogenesis of TB. Our results showed that peoples with *IL-1R psti1970/C* allele are nearly twice more susceptible toTB than those with *IL-1R psti1970/T* allele. We could not confirm that *IL-1R psti1970/C:C* genotype also is positively associated with TB.[40] On the other hand *IL-1â +3962/C* allele showed protective association. Unlike the result reported for Colombian population who showed strong protective association of *IL-1â +3953/T* allele carrying genotypes and TB,[41] and contradictory to the results obtained for Iranian,[42] Cambodian,[43] and population from western region of Gambia[44] who could not confirm any association, we found that people with *IL-1â +3692/T:T* genotype have 4.4 times higher risk to develop TB. Contradictory, *IL-1â +3692/C:T* genotype showed negative (protective) association for TB. Unlike the results of several authors,[40,43] our investigation seems to refute any association between *IL-1Ü* and *IL-1RA* polymorphisms (alleles or genotypes) and TB.

Interleukin 12 (IL-12) as a part of IL-12/IFN-ã axis plays an important role in the host defense against intracellular pathogens like *Mycobacterium tuberculosis.*[45] Although several authors investigated the role of IL-12 polymorphisms and their association with TB, results are still obscure. It has been reported that besides the polymorphism in the promoter region, polymorphism in 3'UTR correlate with the protein secretion.[46] We investigated the possible role of the polymorphisms in the *IL-12B* gene 3' untranslated region (UTR) in the pathogenesis of TB. Our results showed no significant differences of frequency at alleles and genotypes level, suggesting that polymorphism in 3'UTR has no or has negligible effect on the pathogenesis of TB in the population of ethnic Macedonians. These results correlate with others.[47-50]

IFNã is one of the participants in the IL12/IFN-ã axis, besides IL-12, IL-12R and IFNãR. It is the most important Th1 type cytokine involved in the host defense against *M. tuberculosis*[51] via macrophage activation,[52] and plays a major role in defense against viruses and intracellular agents. Many studies indicate that one of the *IFN-ã* gene polymorphisms, the polymorphism *+874 T/A* located in the first intron of the *IFN-ã* gene, correlates with the IFN-ã production. The */A* allele of this polymorphism correlates with low level of IFN-ã after stimulation.[53] We found significant differences not only in *IFNã utr5644 T/A* allele frequency, but in *IFNã utr5644* genotypes containing */A* allele *(/A:A, /A:T*) as well. The *IFNã utr5644/A:A* genotype is positively associated with TB patients with odds ratio of 2.443, meaning that people with *IFNã utr5644/A:A* genotype have 2.4 times bigger risk to develop TB in comparison to people with other genotypes. On the contrary, */A:T* genotype was found to be protective with odds ratio 0.492. Concerning the allele level, negative (protective) association for TB was found for the *IFNã utr5644/T*. This polymorphism was also found to be associated with TB in Spanish,[54] Colombian,[55] Sicilian,[56] South African,[57] Croatian,[58] Hong Kong Chinese[59] and Brazilian,[60] but not in Indonesian,[50] Caucasian American[61] Indian,[62] and West African population.[63,64]

Contradictory with data reported by some authors, who found significant negative association at codon 10 *TGFâ1* polymorphism, /T allele and predomination of */C* allele and */C:C* genotype among patients with pulmonary tuberculosis,[40] in our study we did not find any significant association between TB and *TGF-â1* polymorphisms in codon 10 and codon 25. Similar findings for *TGF-â1* obtain other authors.[55, 65, 66]

Patients with advanced TB have higher levels of serum TNF-Ü than patients with mild tuberculosis or healthy people.[67] Although results of associations between *TNF-Ü* polymorphisms and

susceptibility/resistance to TB have been already studied[68,69] and showed ethnic-specific pattern, they are still questionable. Our results for association between *TNF-Ü -308 A/G* polymorphism and TB correlate with results obtained for Thais,[70] Cambodian,[42, 71] Colombian[55] and population from India,[72] but are apparently in disagreement with those data which find significant association between certain *TNF-Ü-308* genotypes and TB in three ethnic group from Bashkorstan[73] and in Sicilian patients. [74] In contrast to the previously reported results,[70, 72] we could confirm the positive (susceptible) association of *TNF-Ü-238/G* allele for TB. However, we could not confirm the findings of an earlier study from Iran that *TNF -238* genotypes were associated with TB.[40] Only a few authors have investigated the role of other *TNF-Ü* polymorphisms and TB, and found no association between them and TB.[42,70] It remains questionable whether this observed association is solely effect of *TNF-Ü*polymorphisms or association with HLA-Al, B17, B21 and DR7 may play a significant role in pathogenesis of TB.[72]

To our knowledge, this is the first study analyzing the association between IL-2 polymorphisms and TB. In our study we did not find any significant association in *IL-2 -330* and *IL-2 +160* frequencies of alleles, genotypes, haplotypes, or diplotypes.

IL-4 is generally elevated in advanced stages of tuberculosis[75] and by down regulating macrophage activation may determine whether the infection becomes latent or progressive.[76, 77] It also induces production of mucus and hyperplasia of goblet cells[78] in bronchial submucosa. Its gene together with the gene of the IL-13 are located on the chromosome 5q31, only 20 kilobase apart, in the region associated with airway hyper-responsiveness.[79] IL-4 mediates its activity through IL-4 receptor (IL-4RÜ). In this study, we investigated alleles and genotypes of three polymorphisms of *IL-4* (at positions *-1098, -590,* and *-33*), as well as haplotypes and diplotypes of investigated polymorphisms. It has been shown that polymorphism at position *-590* in the *IL-4* promoter region is associated with enhanced *IL-4* promoter strength and altered IL-4 level and activity, and thereby influences the IL-4 dependent events which determine disease progression.[80,81] Our results showed that *IL-4 -1098/G* allele was protective for TB. Frequency analysis of genotypes showed that homozygous genotypes of two *IL-4* polymorphisms (*IL-4 -098/T:T*, and *IL-4 -590/C:C*) were associated with susceptibility for TB, while heterozygous genotypes of the same *IL-4* polymorphisms (*IL-4 -1098/G:T*, and *IL-4 -590/C:T*) showed protective association. Our results obtained for *IL-4 -590* genotypes are not in agreement with those gained for Iranian population[40] and seem to be inverse with the results gained for population of India.[62] We also found that two of the *IL-4* haplotypes (*IL-4/TCT* and *IL-4/TCC*) have susceptible association for TB, as well as protective association with other two haplotypes (*IL-4/GCC* and *IL-4/TTC*). From the diplotypes analysis, we can see that combination of *IL-4* haplotypes *IL-4/TCT:TTT, IL-4/TCC:TTC* and *IL-4/TCC:TCC* were positively associated with TB, and only one *IL-4* dilpotype (*IL-4/GCC:TTC*) was negatively associated with TB. We did not find any significant association between *IL-3 -33* and *IL-4RÜ +1902* frequencies of alleles and genotypes and TB.

Although it is known that IL-6 is important in the initial innate response to *M. tuberculosis*,[82,83] its role in the pathogenesis of TB is still obscure. Human macrophages in response to *M. tuberculosis* infection secrete IL-6,[84] but it is not vital in generation of specific protective immunity.[83] Only a few studies have analyzed the possible relationship between *IL-6* polymorphisms and TB. In this study, the *IL-6 -174 C/G* and *IL-6 nt565 A/G* gene polymorphisms showed no association with tuberculosis. These results are in agreement with other studies,[55] since significant association between TB and *IL-6 -174* genotypes was shown only in Iranian patients.[40]

IL-10 belongs to the group of macrophage-deactivating cytokines, that down regulates Th1-induced response to *M. tuberculosis* and reactivates chronic pulmonary TB in mice.[85, 86] IL10 inhibits IFN-ã production by T cells[87] and counter balanced pro-inflammatory effects of TNF-Ü[88] It has been also shown that IL-10 converts human dendritic cells into macrophage-like cells with increased anti-mycobacterial activity.[89] Hence, IL-10 may play an

important role in the pathogenesis of TB. We investigated the associations between three SNPs in the *IL-10* gene promoter region *(-1082 A/G, -819 C/T,* and *-592 A/C*) and TB. When studied independently there was no significant association between all three investigated *IL-10* alleles and TB. However, analysis of genotypes showed that */G:G* genotype (homozygous *G* allele) was susceptible to TB. Patients with this genotype have 2.5 times bigger risk to develop TB. The same risk for developing TB have the patients with *IL-10/GCC:GCC* diplotype. This risk became much higher in patients with *IL-10/ATC* haplotype (odds ratio=8.164) and *IL-10/ATC:GCC* combination of haplotypes (diplotype or haplotype zygozity) (odds ratio 8.366). Data showed that *IL-10 -1082/GG* haplotype[90] and *IL-10/GCC* haplotype correlates with higher level of IL-10 after stimulation, which leads to the suppression of IFN-ã, and hence favours development/relapse of TB.[59,90] It was found that */ GCC* haplotype was associated more with the development of relapse/extra-pulmonary TB, than with the susceptibility to TB.[59, 91] Previous investigations have showed that *IL-10 1082 A/G* polymorphism was associated with TB in the Hong Kong Chinese,[59] Colombian,[55] Spanish,[92] Turkish,[93] Cambodian,[42] but not in the Gambian[43] and Spanish population.[92] In Korean populations not *IL-10 -1082A/G* polymorphism, but *IL-10 -592 A/C* promoters polymorphism was found to have significant association with TB.[94] Some authors claimed that not solely effect of *IL-10* polymorphisms, but the combination of certain *IL-10* and *TNF-Ü* genotypes might influence the outcome of inflammation response against TB.[74]

Although mechanisms associated with polymorphisms that lead to the changes in gene expression are still poorly understood, it is well-known that TB is partly under polygenic control. The genetic components that play role in host defense to TB encompass not only multiple alleles located on different genes and even on different chromosomes, but also gene-environment interaction as well. Still, it is not very well known whether polymorphisms itself contribute to susceptibility/protection to TB or their linkage disequilibrium with an unknown disease susceptibility allele. Many of these polymorphisms occur in ethnic-specific patterns. Identification of these genes and these kinds of interactions are of great importance in clarifying the role of genetic factors in pathogenesis of TB and may lead to new ways of treatment or prophylaxis.

In conclusion, we confirm that some cytokine polymorphisms contribute to susceptibility/protection to tuberculosis in Macedonians.

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Original Article

ASSESSMENT OF LONG TERM STATUS OF SPUTUM POSITIVE PULMONARY TB PATIENTS SUCCESSFULLY TREATED WITH SHORT COURSE CHEMOTHERAPY

V.V. Banu Rekha, Rajeswari Ramachandran, K.V. Kuppu Rao, Fathima Rahman, A.R. Adhilakshmi, D. Kalaiselvi, P. Murugesan, V. Sundaram and P.R. Narayanan

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Summary

Background: Long term status of pulmonary tuberculosis (PTB) patients treated with short course chemotherapy (SCC) regimens remains unknown.

***Objective*:** To assess the clinical, bacteriological, radiological status and health related quality of life (HRQoL) of PTB patients 14 -18 years after successful treatment with SCC.

Methodology: In a cross-sectional study, cured PTB patients treated during 1986 – 1990 at the Tuberculosis Research Centre (TRC) were investigated for their current health status including pulmonary function tests (PFT). The St Georges respiratory questionnaire **(**SGRQ) was used to assess the HRQoL

***Results*:** The mean period after treatment completion for the 363 eligible participants was 16.5yrs (range 14-18 yrs, 84% coverage) ; 25 (7 %) had been re-treated and 52 (14%) died. Among the investigated, 58 (29%) had persistent respiratory symptoms; 170(86%) had radiological sequelae but none had active disease. Abnormal PFT was observed in 96 (65%) with predominantly restrictive type of disease in 66(45%). The SGRQ scores for activity and impact were high implying impairment in HRQoL.

Conclusion: Assessment of long term status of cured PTB patients showed an impairment of lung functions and HRQoL highlighting the need to address these issues in the management of TB that may provide added value to patient care.

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Key Words: Long term assessment. SCC, PTB, PFT, SGRQ

INTRODUCTION

Standardised and directly observed six month regimen is currently recommended for the treatment of Pulmonary Tuberculosis (PTB). The main objective of treatment in PTB is to achieve high bacteriological cure rates. Though the patients are cured of the disease, the information on the long term status of the patients successfully treated with short course chemotherapy (SCC) regimens remains largely unknown. Studies have documented that about 1/3rd of the cured PTB patients do have respiratory complaints at the end of treatment seeking medical care[1,2]. The morbidity in PTB patients while on treatment or at the end of treatment has been reported using subjective (quality of life) as well as objective (pulmonary function tests) measurements[1-7]. However, the long term status of cured PTB patients has not been studied. This information is essential to quantify the impact of disease for appropriate early interventions in the management of patients with PTB. Hence the Tuberculosis Research Centre (TRC) undertook a study to assess the clinical, bacteriological and radiological status of PTB patients14 -18 years after successful completion of treatment with SCC .The aim of the present communication is to analyze the morbidity in these patients.

METHODOLOGY

Setting

TRC has been undertaking randomized controlled clinical trials in the treatment of pulmonary and extra-pulmonary forms of TB since 1956 with the objective of evolving suitable chemotherapeutic regimens in patients suffering from TB. The patients enrolled in the trial are followed up bacteriologically and radiologically for a maximum period of five years.

Tuberculosis Research Centre, Chennai.

Correspondence: Dr. Rajaswari Ramachandran, Formerly Deputy Director, Senior Grade, Tuberculosis Research Centre, Mayor V.R. Ramanathan Road, Chetput, Chennai-600 031.Phone: 91 44 29369613; Mobile 9444057486; e-mail: rajerama@yahoo.com

Study population

This cross-sectional study was conducted at TRC, Chennai, during August 2004 – 2005. The study population included patients enrolled during 1986-1990 and had bacteriological quiescence (ie. culture negative) at the 60th month of follow-up. These were new PTB patients (≤1 month of previous anti-tuberculosis drugs) who were treated with first line anti-tuberculosis drugs namely isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E) in one of the following SCC regimens which included six month intermittent ($2EHRZ_2/4EHR_2$, $2HRZ_2/4RH_2$) or nine month daily (2EHRZ/ 6EH) regimens.

All efforts were made to interview the patients during home visits or over the telephone. In case of reported death of the patient, a doctor's visit was made to probe the reasons for death (verbal autopsy)

When the patient attended the centre, a detailed history which included re-treatment during the follow-up period was elicited, after obtaining informed consent. Information was also obtained on habits which included smoking and alcoholism, respiratory symptoms and co-morbid conditions like diabetes, hypertension, bronchial asthma and cardiac problem. The blood pressure, weight and height were recorded and a general and systemic examination was performed. Other investigations included postero-anterior chest radiography, two sputum examination by smear and culture for tubercle bacilli, 12 lead Electrocardiogram (ECG) and Pulmonary function test (PFT) by Spirometry. The quality of life was assessed by a trained social worker using the St. Georges respiratory questionnaire (SGRQ).

Bacteriology: Sputum smears were examined for AFB by fluorescence microscopy.[8] and the specimens were processed by modified Petroff's method and cultured on the LJ medium.[9] All positive cultures were subjected to identification tests for *Mycobacterium tuberculosis* and to drug susceptibility tests using the minimal inhibitory concentration method for isoniazid and rifampicin[10,11].

Radiology: The chest x-rays were read by a panel of three doctors. The following observations were recorded namely a) the extent of lung involvement which was documented as unilateral or bilateral, b) the number of zones involved (minimum one-zone to maximum six -zones and c) presence or absence of cavitation[12].

Electrocardiogam (ECG): A 12 lead ECG was recorded for the patients and independently read by the cardiologist. The criteria laid down by WHO (1961) were used for the ECG based diagnosis of cor-pulmonale[13].

Pulmonary function testing (PFT) – PFT was performed according to the techniques mentioned in the operating manual of the spirometry (sensormedix) with special reference to American Thoracic society of Standardization of spirometry.[14] The parameters measured in spirometry include Forced Vital Capacity (FVC), Forced expiratory volume in 1 second (FEV_1), ratio of FEV_1 to FVC (FEV_1%). For assessing chronic changes, the observed values of FVC, FEV_1, and FEV_1% were expressed as a percentage of the predicted values by using the regression equation developed by Vijayan[15]. Lung function impairments were classified according to the above mentioned parameters as normal/restrictive disease/obstructive disease/ combined disease[14] .

St Georges respiratory questionnaire (SGRQ): In the present study. SGRQ, a standardized, airways disease specific respiratory questionnaire, was used to measure health related quality of life (HRQoL)[16, 17] This health status instrument consisting of 50 items is grouped under three components namely symptoms (8 items) which measures respiratory symptoms, activity (16 items) which measures impairment of mobility or physical activity and impact (26 items) which measures the psychosocial impact of disease). This tool has been validated for use in chronic pulmonary diseases including TB in many countries including India[18-23]. SGRQ scores were calculated using score calculation algorithms and missing data imputation recommended by its developer (P.W. Jones, St Georges Hospital Medical School, London, UK) Scores ranging from 0 to

100 are calculated for each component, as well as a total score which summarizes the responses to all items. Higher scores correspond to worse health-related quality of life.

Analysis

The data were analyzed using a statistical software package (SPSS, version 13 for Windows; Inc; Chicago IL). The SGRQ was scored with the scoring calculator (Excel-based) provided with the instrument. For each SGRQ scale, distribution characteristics, the percentage of patients with missing information and reliability estimates were calculated. Analysis of variance and t-test were used to compare SGRQ scores between groups defined by functional characteristics.

RESULTS

There were 455 patients who were treated between 1986-1990 and for the present study 92 were excluded since 63 were re-treated for relapse, seven had defaulted, 20 died of non-tuberculous cause and in two patients treatment was changed. Hence 363 were eligible for the present study (Figure). This included 163 and 200 patients who were treated with daily and intermittent regimens of

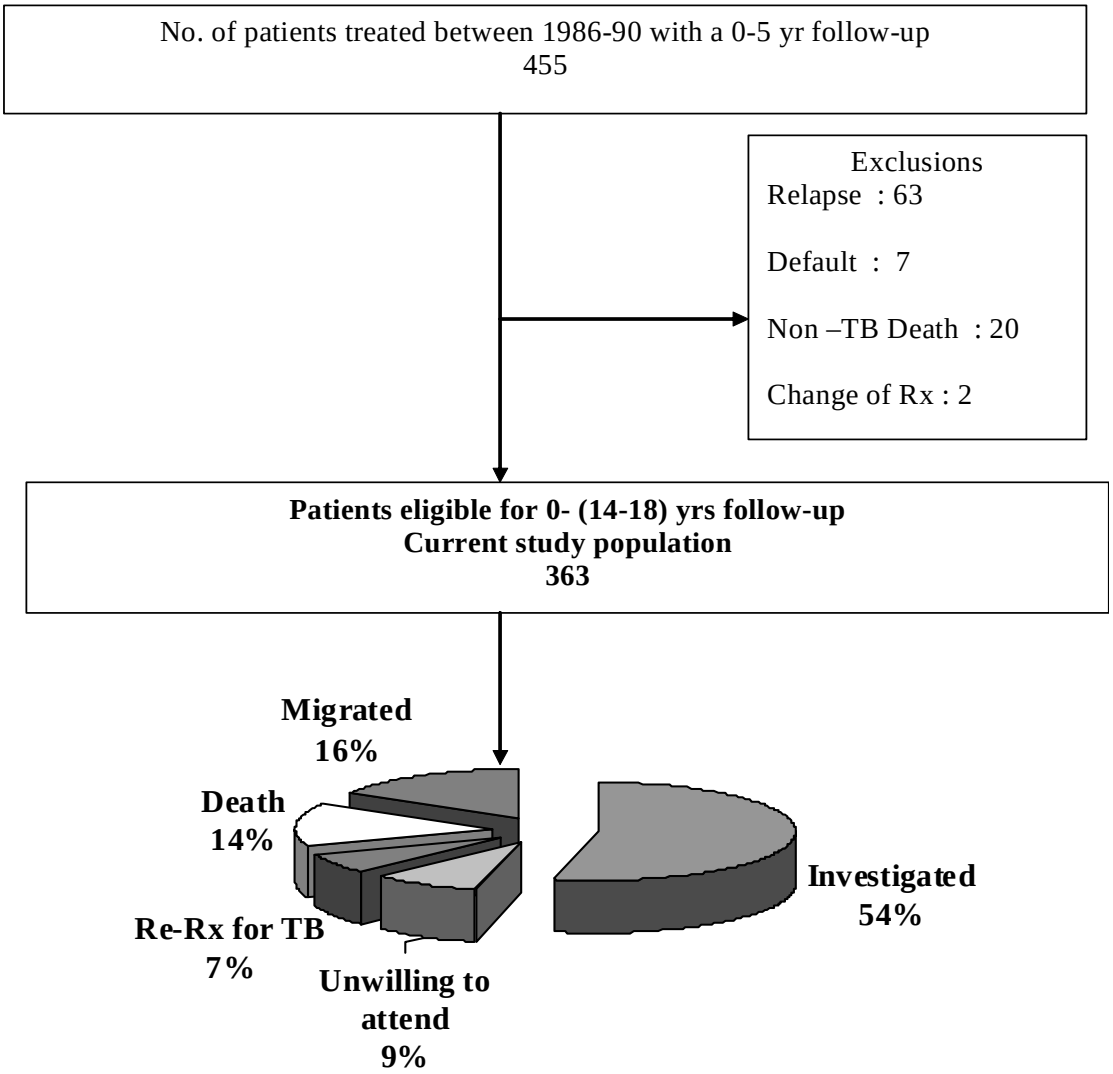

Figure: Status of pulmonary TB patients treated with SCC after a period of 14-18 years (n = 363)

SCC respectively. The mean period after treatment completion for the 363 eligible participants was 16.5yrs (range 14-18 years).There were 59 (16%) patients who had migrated and information is available for the remaining, giving a coverage of 84%. There were 25 (7%) who had been re-treated and 52 (14%) patients who had died. Among the 227 patients who were available, 198 (87%) attended our centre for investigations to assess the current health status.

Re-treatment: Among the 25 (7%) patients who were retreated, two were re-treated for extra-pulmonary TB (Brain tuberculoma, TB lymphadenitis).

Mortality: A verbal autopsy of the 52 (14%) patients who expired including five females revealed no deaths due to active TB. The mean age of these patients at the time of death was 50yrs. The cause of death could be attributed to respiratory pathology in 15 (29%) patients as the history of events preceding death were repeated attacks of cough and breathlessness necessitating frequent hospitalization or medications.

Profile of patients who were investigated: The mean age of the 198 patients who were investigated was 46yrs (range 27-73years). Majority 124 (63%) were males among whom 50% were smokers. (Table-1).

Clinical: Respiratory complaints were reported by 58 (29%) of the patients, mainly of frequent cough and/or breathlessness.

Bacteriology: Sputum smear and culture negativity was seen in 193 (97%) of the study participants.

Table 1. Characteristics of the patients who were investigated (n= 198)

Parameters		n	%
Sex	Male	124	63
Habits	Smoking	62	50
	Alcohol	47	38
Respiratory symptoms free		140	71
Bacteriology	AFB smear negative	193	97
	M.tb culture negative	196	99
Chest X-ray	Normal	28	14
	Abnormal	170	86
	Fibrosis	62	36
	Calcification	41	24
	Fibrosis & Calcification	59	35
	Others	8	5
	Zones ≤ 2	71	42
	>2	99	58
ECG			
	Normal	160	81
	Cor-pulmonale	21	11
	Others	13	6
	Un co-operative	4	2

Table 2: Types of abnormality in lung function in 148 investigated patients treated 14-18 years ago with SCC

Type of disease	Males		Females		Total	
	n	%	n	%	n	%
Normal	37	38	15	30	52	35
Restrictive disease	34	35	32	64	66	45
Obstructive disease	7	7	0	0	7	5
Combined disease	20	20	3	6	23	16
Total	98	100	50	100	148	100

Among the five patients who had positive smears, three patients were culture negative while two patients' cultures grew *Mycobacterium kansasii*.

Radiology: There were 28 (14%) patients with normal chest radiography while others had abnormality predominantly of fibrosis and or calcification.

Spirometry: The pulmonary function tests were performed in 148 (75%) patients who were willing to participate which included 98 males and 50 females (Table-2). The spirometry was normal for 52 (35%) of the participants. The predominant lung function impairment was restrictive type of disease in 66 (45%) patients and this was observed more in females - 32 (64%). Obstructive and combined type of lung function impairments were predominant in males. The difference in lung function impairments between the smokers and non-smokers was not statistically significant.

Electrocardiogram (ECG): Features suggestive of cor-pulmonale was observed in 21(11%) of the patients. The abnormal ECG changes observed in the remaining 14(7%) patients were that of left ventricular hypertrophy or heart rate disturbances like sinus bradycardia or tachycardia.

SGRQ: Tables 3 and 4 describe mean SGRQ scale scores in study population according to sex, smoking behaviour, ECG, chest X-ray and FEV_1% predicted.

Table 3: Distribution characteristics and reliability estimates SGRQ scales in the study population weighted sample (n=198)

	Symptoms	Activity	Impact	Overall
Mean Normal values*	12	9	2	6
Mean observed values	16.46	28.95	14.11	19.60
SD	18.63	23.19	23.96	20.65
Observed range	0-79.53	0-100	0-92.5	0-83.65
% with missing data	1.5	3.1	4.6	5.1

***** Reference No: 26

Table 4: Mean SGRQ scale scores by demographical and clinical characteristics of the study population.

		Symptom	Activity	Impact	Overall
Mean Normal values*		12	9	2	6
Sex	Male	15.10	24.55	10.95	16.43
	Female	18.78	36.27	19.24	24.72
	p value	NS	0.001	0.03	0.01
Male	Smoker	19.63	30.37	14.56	21.00
	Non-smoker	11.45	19.98	8.07	12.85
	p value	0.02	0.02	NS	0.03
Chest Radiography	Abnormal	17.46	28.72	15.05	20.23
	Normal	10.58	30.29	8.79	16.12
	p value	0.01	NS	NS	NS
No. of zones involved Chest radiography	≤ 2	15.07	29.34	12.76	19.03
	>2	17.90	28.56	15.46	20.17
	p value	NS	NS	NS	NS
ECG	Abnormal	20.85	36.36	21.08	26.17
	Normal	15.31	27.34	12.19	17.89
	p value	NS	0.03	NS	NS
PFT (n= 148)					
FEV_1 % predicted	≤80%	19.35	28.98	14.10	19.97
	>80%	15.06	28.15	13.28	18.57
	p value	NS	NS	NS	NS

* Reference No: 26

The mean SGRQ scores for symptom, activity and impact including overall scores were high (increased impairment) among this group of patients as compared to that of general population. The overall scores were significantly higher for females (p=0.01) and smokers (p=0.03). However, abnormal chest X-ray, ECG and FEV_1% predicted did not correlate significantly with high SGRQ scores.

The above mentioned clinical, bacteriological, radiological, spirometry and ECG findings were not different from those who were treated with daily or intermittent SCC regimens.

DISCUSSION

To our knowledge, this study is the first of its kind to assess the long term status of PTB patients, 14-18 years after treatment with SCC regimens. There were 58 (29%) participants who had persistent respiratory symptoms and 170 (86%) with radiological sequelae. Lung functions impairments were present in 96 (65%) while ECG showed evidence of cor-pulmonale in 21(11%). Thus majority of the cured PTB patients had significant morbidity as evidenced by impairment in pulmonary functions and high SGRQ scores. This

study highlights the presence of respiratory system related morbidity among the microbiologically cured PTB patients.

Persistent respiratory symptoms observed in approximately 1/3rd of the patients in the present study is an important cause of concern. Studies have documented that cured PTB patients continue to have respiratory symptoms (30-47%) at the end of treatment, (40%) after one year of treatment and (15.9%) after two and a half years after treatment[1,2,5,24] In another study 14% of treated PTB patients continued to attend the out-patient department for more than five years for respiratory complaints.[25] Since all the investigated patients in the present study were culture negative for *Mycobacterium tuberculosis*, there is a need to educate the patients that persistence of symptoms is not synonymous with reactivation of the disease.

The respiratory disease specific SGRQ scores were higher in this cohort of patients which implies an impairment in the quality of life which is in conformity with other studies done among PTB patients[18,19]. As data pertaining to SGRQ scores in general population in India is not available, the normative values for a general population studied in Spain recommended in SGRQ manual is used for comparison. The symptom, activity and impact scores observed in our study after a mean period of 16.5 years after successful treatment for TB were 16, 28 and 14 respectively as compared to general population scores of 12, nine and two on a scale of 100[26]. These findings suggest that the scores of the treated PTB patients were high (indicating worse status) when compared to the general population. Similar to earlier SGRQ studies in PTB patients, the score for impact was lower than that of symptom and activity[18,19] The scores were less when compared to patients with other respiratory diseases like interstitial lung disease, COPD or bronchiectasis[20,21,22]. These findings suggest that HRQoL among treated PTB patients was suboptimal when compared to the general population, but better when compared to other respiratory diseases.

Generally the observed SGRQ scores are higher for females[27]. In this cohort the female participants had a significantly higher scores in SGRQ suggestive of impairment of HRQoL with reference to activity and impact of disease as compared to males. An earlier study done on PTB patients while on treatment showed no gender differences in the SGRQ scores[18]. Among the males, in smokers SGRQ showed significant impairment of quality of life emphasizing the need to create an awareness about smoking cessation among treated TB patients.

Lung function impairments after TB is an unrecognized cause of chronic lung disease worldwide[7]. In the present study PFT was done in 75% of the patients. The predominant lung function patterns observed in these patients were restrictive and combined type similar to other published studies[6,7]. Our findings, similar to a previous reports have shown that females had more restrictive type of disease compared to males[3]. A previous study among treated PTB patients from our centre showed that the main ventilatory type of abnormality was restrictive in nature where as a study from Pretoria concluded that equal number of patients had restrictive/ obstructive type of disease[6,28]. However, a study from South Africa which was done at an average of 16 years after TB treatment showed that 68% of the patients had chronic obstructive lung disease[29]. Impairments in lung function in the present study were not significantly high among smokers. The published studies on smoking and lung function impairments in PTB patients are varied[19,30]. The patients with a FEV_1% Pred. of $\leq$80% had high SGRQ scores implying poor quality of life though it did not attain statistical significance

We observed a mortality of 14% in this cohort of patients. A study done among PTB patients irrespective of being treated has shown a mortality rate of 24% and lung function impairment as the probable cause of death in 15% after a duration of 10 years[30]. Although 15 patients in our study had frequent respiratory complaints or hospitalization among those who expired, it was not possible to conclude that TB sequelae was the probable cause of death.

The high coverage observed in our study could be attributed to the stringent efforts in screening for domiciliary stability for enrollment of patients to our controlled clinical trials. We observed no difference in the profile of patients treated with daily or intermittent anti-tuberculosis therapy. This highlights the fact that intermittent chemotherapy is as effective as daily pertaining to the long term impact of pulmonary TB.

The major limitation of this study is that the observations made need not be confined to TB sequelae alone and could be due to the aging and or health related factors of the study population. The lack of concurrent age-sex matched controls coupled with the lack of baseline investigations for pulmonary functions and assessment of quality of life is also another major limitation.

Despite the above mentioned limitations, this study has shown that treated and cured PTB patients do suffer from long term morbidity. India is a high burden country for TB and there is an active and well structured Revised National TB Control Programme (RNTCP) operating successfully covering the entire population since March 2006. The RNTCP has treated more than 8.1 million patients, and thereby prevented almost 1.46 million TB deaths.[31] As the programme matures, its level of sophistication will undoubtedly increase.[1] **From the findings of this study it appears that the providers and programme managers have a responsibility to recognize and address the long term impact of TB even after patients are cured. Addressing issues relevant to the quality of life and considering measures for pulmonary rehabilitation will provide added value to the programme.**

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Original article

MYCOBACTERIAL ES-31 SERINE PROTEASE – A BIOMARKER FOR MYCOBACTERIUM TUBERCULOSIS – A PRELIMINARY REPORT*

M. Anindita[1], V. Upadhye[1], D. Thamke[2], D.K. Mendiratta[2] and B.C. Harinath [1]

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Summary: There is a need for simple and reliable method to identify *Mycobacterium tuberculosis* from AFB smear positive cases. Utility of mycobacterial ES-31 serine protease as a marker to detect *Mycobacterium tuberculosis* bacilli was explored using Fluorescein isothiocyanate conjugated anti-ES-31 serine protease antibody. The presence of ES-31 serine protease in bacilli was indicated by green fluorescence on the cell surface. Green fluorescence was observed with *M.tb*. H_{37}Ra bacilli and *M.tb*. H_{37}Rv bacilli while no Fluorescence was observed with *M. chelonae*, *Nocardia farcinicum* as well as in *E. coli* showing the usefulness of ES-31 serine protease as a marker for identification of mycobacterium tubercle bacilli in cultures. **[*Indian J Tuberc 2009; 56:141-143*]**

***Key words*:** Mycobacterial ES-31 serine protease, Immunofluorescence, Non-tuberculous mycobacteria

INTRODUCTION

Detection of presence of acid fast bacilli in clinical specimens is not sufficient and requires labour intensive biochemical methods for further identification of Mycobacterial species. Many people harbour NTM in their respiratory secretions without any symptoms. The immunosuppressed individuals infected by human immunodeficiency virus (HIV) infection have become most significant risk factor for disseminated NTM disease and of these, 95% are due to *Mycobacterium avium complex* (MAC). Clinical differentiation of *M. tb*. infection and other mycobacteriosis is difficult due to overlapping domain of symptoms. Differentiation of *M.tb*. from NTM depends upon use of time consuming conventional morphological and biochemical tests or expensive genotypic methods[1]. Thus, there is a need for exploration of newer simple and rapid methods to distinguish *M. tb*. from NTM.

In earlier studies from our laboratory, we have reported diagnostic importance of ES-31 protein antigen, a serine protease in the diagnosis of pulmonary tuberculosis and TB in HIV-TB co-infection[2,3]. In this study we explored the utility of mycobacterial ES-31 serine protease as a marker, if any, to identify tubercle bacilli using Fluorescein isothiocyanate(FITC) conjugated anti – ES-31 serine protease antibody.

Bacilli used in the present study include *M. tb*. H_{37}Ra bacilli and *M. tb*. H_{37}Rv bacilli; NTM such as *Mycobacterium chelonae (M.* chelonae), *Nocardia farcinicum* and *Escherichia coli (E. coli)*.

Isolation of anti ES-31 antigen and its antibody and conjugation with Fluorescein isothiocyanate (FITC):

M. tb. H_{37}Ra detergent soluble sonicate (DSS) antigen, was prepared from *M.tb*. H_{37}Ra bacilli. Briefly, bacilli were 5% phenol inactivated in 0.5M phosphate buffer (PBS, pH7.2) and incubated with sodium dodecyl sulphate (SDS) extraction buffer. The supernatant was dialysed against 0.01M PBS, pH 7.2 and used as an antigen source. Anti-DSS IgG antibodies were raised in goat by immunizing intramuscularly with 500 µg protein/mL DSS antigen with 1 ml Freund's incomplete adjuvant on days 0, 20, 33 and 45. Immune sera were collected on days 32, 44, 57, 60 and thereafter fortnightly and anti-

1. Jamnalal Bajaj Tropical Disease Research Centre, Sevagram, Wardha
2. Department of Microbiology, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha
* **As a part of the research project financially supported by Tuberculosis Association of India.**
***Correspondence*:** Dr. B. C. Harinath, Director, JB Tropical Disease Research Centre and Department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences, Sevagram - 442 102 Wardha (Maharashtra); Tele Fax: (07152) 284038; E-mail: bch@jbtdrc.org, bc_harinath@yahoo.com

SDS IgG was isolated by 33% saturation with ammonium sulphate under ice, followed by diethyl aminoethyl-cellulose ion exchange column chromatography as described earlier[4]. ES-31 antibodies were isolated from anti-DSS IgG by affinity chromatography using ES-31 antigen coupled Sepharose-4B column. Anti ES-31 antibody – FITC conjugate was prepared as follows:

In brief, 2.5 mg anti-ES 31 antibody diluted to 1ml with 0.145M sodium chloride solution. 12.5 µg FITC / mg protein (pH 9.5) was added and the mixture incubated at 25^0C for 45 min. Conjugate mixture was applied through Sephadex G-25 column and eluted with 0.01M PBS (pH 7.2) at a flow rate of about 30 ml / min. First eluted yellow coloured fractions were collected which contained anti-ES-31 antibody conjugated with FITC. FITC conjugate was concentrated by ultra-membrane filtration and stored at 4^0C.

Immunofluorescence assay for detection of Serine protease

The detection of ES-31 serine protease in bacilli was performed using FITC labelled anti- ES-31 serine protease antibodies. In brief, a loopful of bacilli was incubated with 100 µg FITC conjugated antibody in a conical vial for 1 hour at 37^0C. Bacilli were washed twice with 0.05 M PBS and mounted using mounting medium (50 % glycerol, 50% PBS and 0.1% Sodium azide). Bacilli were observed under Nikon Labophot Microscope with episcopic fluorescent attachment for fluorescence. In the present study, each sample had been analyzed in duplicate.

RESULTS AND DISCUSSION

The presence of ES-31 antigen in bacilli using FITC labelled anti- antibody was indicated by presence of green fluorescence on cell surface when observed under microscope. Green fluorescence was observed with *M. tb*. H_{37}Ra bacilli and *M. tb*. H_{37}Rv bacilli (Fig. A), while no Fluorescence was observed with *M. chelonae*, *Nocardia farcinium* (Fig. B) as well as in *E. coli*, indicating that ES-31 serine protease is specific for *mycobacterium tuberculosis*.

Early identification of TB infection helps in the prompt initiation of drug treatment and better management of disease. In the present study, immunofluorescence method was explored using FITC labelled anti ES-31 serine protease antibody to study the usefulness of ES-31 antigen as a biomarker. Presence of ES-31 serine protease was observed to distinguish *M.tb*. from NTM. It is simple

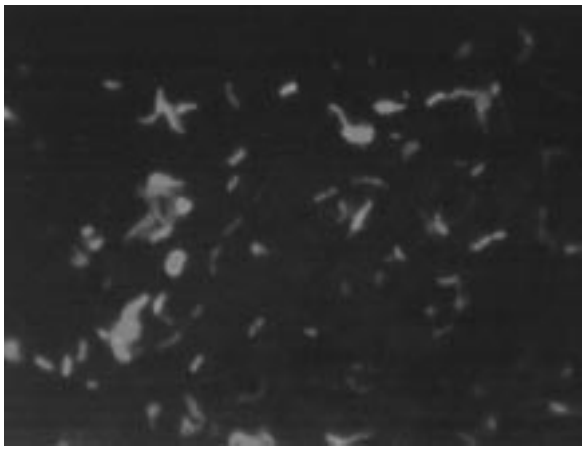

Fig A: Green fluorescence with M.tb H_{37}Rv bacilli showing the presence of ES-31 serine protease on the cell surface.

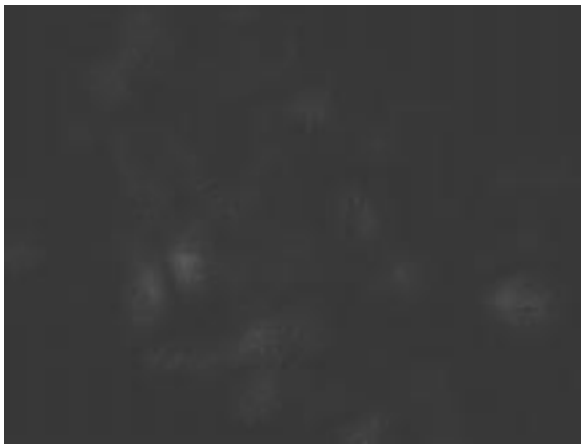

Fig B: Absence of FITC stain on non-tubercle mycobacterial bacilli (*Nocardia farcinica*)

and gives results only within two hours, however it requires fluorescence microscope.

Currently, differentiation of *M.tb*. from NTM depends upon use of labour intensive biochemical or sophisticated phenotypic and genotypic methods. *M.tb*. has ability of niacin production. Konno first devised the standard niacin test, which was modified by Runyon et al for differentiation of *M. tb*. from NTM which is being routinely used in the hospitals[5]. Discrimination of *M. tb*. from NTM is very important in view of clinical management of case as treatment of NTM infections differs in several facets. It depends upon the infecting organism and the severity of the infection. Hence *M.tb*. should be differentiated from NTM so that proper treatment may be given.

In this preliminary study, it has been shown that SEVA TB ES-31 Serine protease can be used as a biomarker for identification of *M. tb*. using FITC labelled antibody. Further enzyme immunoassay or rapid immunochromatographic test using peroxidase conjugated anti ES-31 antibody may be adapted for diagnosis of TB, in smaller laboratories.

ACKNOWLEDGEMENTS

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Original Article

COMPARING OUTCOMES IN NEW PULMONARY SPUTUM POSITIVE AND SPUTUM NEGATIVE CASES UNDER RNTCP IN RURAL INDIA

Abhijit Mukherjee[1], Rupak Singla[2] and Indranil Saha[3]

(Received on 2.12.2008; Accepted on 18.6.2009)

Summary

Setting: The study was carried out at the Bagula TU, Nadia, West Bengal, India.

Objective: To find out the treatment outcomes of new smear negative cases, in low HIV prevalence population, and to compare the results with new smear positive cases in the same population.

Design: It was a retrospective record based study. All patients registered between January 1999 and June 2005 were divided into new smear positive and new smear negative groups and the difference in the outcomes analysed.

Results: Favourable outcome was less in new smear negative cases, compared to new smear positive (84% vs. 86%, p= 0.002).Death and default were more in new smear negative cases, compared to new smear positive (death: 6.8% vs. 3.7%; default: 6.02% vs. 4.18%), ($p < 0.05$). Failure and transferred out were non- significantly higher in new smear positive group.

Conclusions: Smear negative patients had a worse treatment outcome compared to smear positive patients including lower favourable outcomes and higher deaths and defaults. The possible reasons need to be explored and corrective actions need to be taken accordingly. ***[Indian J Tuberc 2009; 56:144-150]***

Key Words: Tuberculosis, Treatment outcome, RNTCP

INTRODUCTION

Under the RNTCP, new pulmonary tuberculosis patients are classified on the basis of the presence of acid fast bacilli in their smears. The demonstration of AFB in the smear of patients with tuberculosis depends on the presence of 10^4 bacilli per ml of sputum. Smear negative patients, having a lesser bacterial load in their sputum, are also started on ATD depending on the presence of clinical and radiological evidence of tuberculosis.

During the initial years following the implementation of the Revised National Tuberculosis Control Programme (RNTCP), more stress was given to the areas of sputum positive case finding and cure of 85% of the detected cases. With the success achievement of the initial goals, the time has come to focus on another important sub-group of pulmonary tuberculosis patients, the sputum smear negatives.

Although studies on smear positive tuberculosis are abundant in the literature, there is very little work on smear negative cases in areas with low prevalence of HIV, the areas that are even now more common in India.

The present study was undertaken to find out the different treatment outcomes of new sputum smear negative cases, in a low HIV prevalence population and to compare the results with new sputum smear positive cases in the same population .

MATERIAL AND METHODS

It was a retrospective record based study, carried out at the Bagula Tuberculosis Unit (TU), Nadia, West Bengal. The TU caters to a population of approximately 0.5 million; where most of the people belong to the lower socio-economic status. The Bagula TU has five Designated Microscopy

State Tuberculosis Demonstration and Training Centre, Medical College, Kolkata.

1. Medical Officer, State Tuberculosis Demonstration and Training Cengtre, Medical College, Kolkata.
2. Head, Department of TB & Respiratory Diseases, LRS Institute of TB & Respiratory Diseases, New Delhi.
3. Assistant Professor, Department of Community Medicine, R.G. Kar Medical College & Hospital, Kolkata.

Correspondence: Dr. Abhijit Mukherjee, 34, S.N. Banerjee Road, New Barrackpore, Kolkata-700 131. e-mail: drabijit71@gmail.com. Tel No. +91 9433187412,

Centres (DMC) manned by five trained laboratory technicians, supervised by a Senior Tuberculosis Laboratory Supervisor (STLS). A total of 2884 patients registered between January 1999 and June 2005 were evaluated for the study. 14 patients with incomplete records were excluded.

Diagnosis, classification and chemotherapy were done and the outcomes following treatment were noted as per the under mentioned RNTCP guidelines[1].

(a) **New**: A patient who has never had treatment for tuberculosis or has taken anti- tuberculosis drugs for less than one month.

(b) **Relapse**: A patient declared cured of TB by a physician, but who reports back to the health service and is found to be bacteriologically positive.

(c) **Failure**: Smear-positive patient who is smear-positive at five months or more after starting treatment. Failure also includes a patient who was initially smear negative but who becomes smear positive during treatment.

(d)**Treatment after default (TAD)**: A patient who received anti-tuberculosis treatment for one month or more from any source and who returns to treatment after having defaulted, i.e., not taken anti-TB drugs consecutively for two months or more.

(e) **Cured**: Initially smear-positive patient who has completed treatment and had negative sputum smear results, on at least two occasions, one of which was at completion of treatment.

(f) **Treatment Completed**: Sputum smear-positive case who has completed treatment, with negative smears at the end of the initial phase but none at the end of treatment.

Or: Sputum smear-negative TB patient who has received a full course of treatment and has not become smear-positive during or at the end of treatment.

(g) **Chronic**: A patient who remains smear-positive after completing a re-treatment regimen.

(h) **Died**: Patient who died during treatment, regardless of cause.

(i) **Default**: A patient who, at any time after registration, has not taken anti-TB drugs for two months or more consecutively.

(j) **Transferred Out**: A patient who has been transferred to another Tuberculosis Unit/District and his/her treatment results are not known.

(k) **Favourable Outcome**: Favourable outcome was defined as cured and treatment completed combined.

(l) **Unfavourable Outcome**: Died, default, failure and chronic cases together are taken together as unfavourable outcome.

Difference between two means and two proportions were tested by student t test and z test for proportion respectively.

RESULTS

At first, total patients were divided into two groups i.e. new sputum smear positive and new sputum smear negative. A total of 1458 new smear positive and 1412 new smear negative cases were registered. In both the cases proportion of male patients were more, compared to female. Proportion of males in new smear positive cases were significantly higher (72.8% vs. 67.0%; $z=3.37$, $p < 0.01$) than new smear negative cases. Mean age of new smear negative cases was significantly lower than new smear positive cases (39.6 ±17.0 yrs vs. 44.4 ± 18.5 yrs) (Table 1).

Favourable outcome was more in new sputum positive cases, compared to new sputum negative cases (88.06% vs. 84.13%), that too was statistically significant ($p = 0.002$). Out of unfavourable outcomes, death and default were more in new smear negative cases, compared to new smear positive (death: 6.8% vs. 3.7%; default: 6.02% vs. 4.18%) and in both the situations the difference was statistically significant ($p < 0.05$). However, failure and transferred out were non-

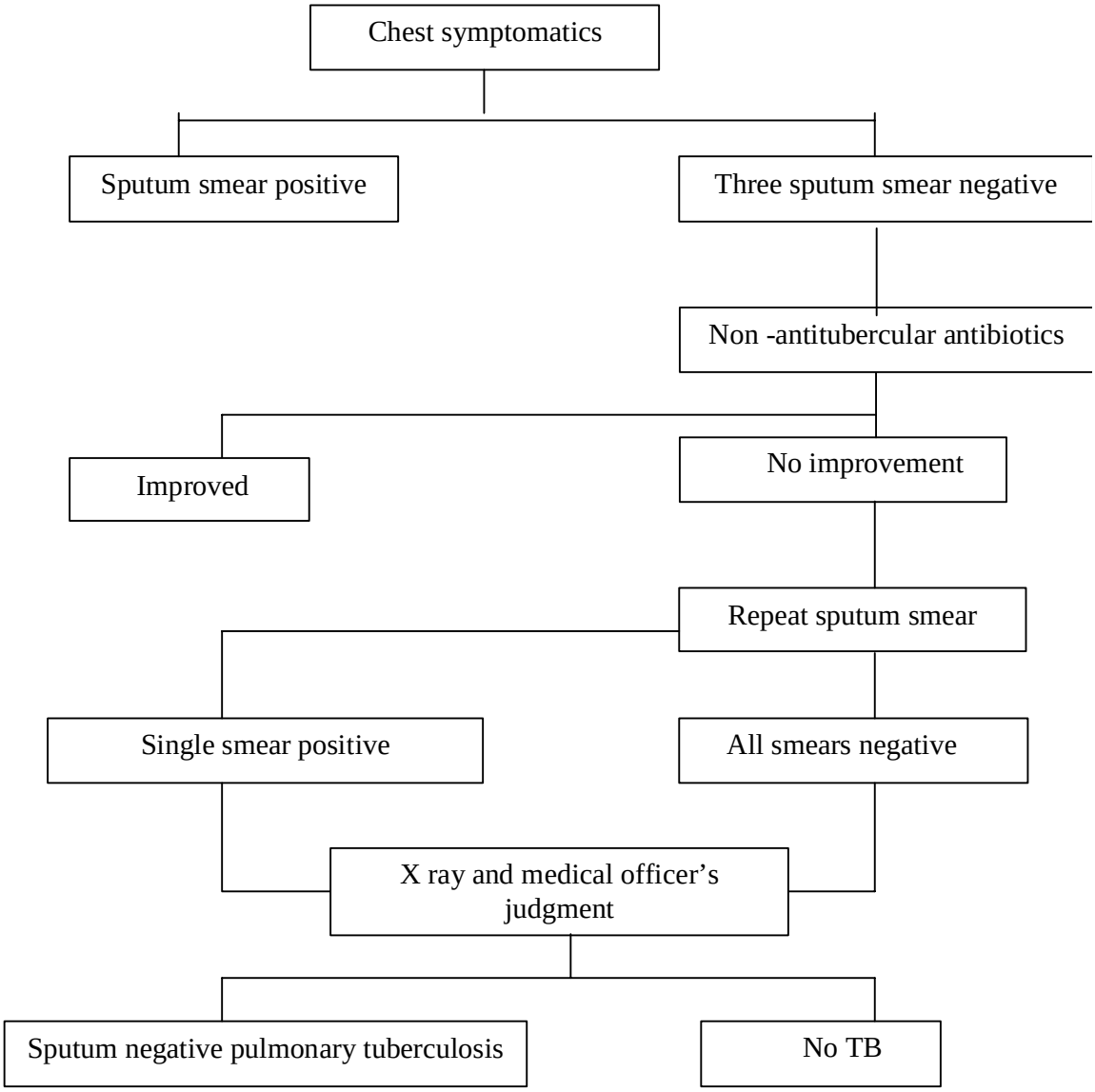

Figure: WHO guidelines for the diagnosis of smear negative tuberculosis.

Table 1. Demographic characteristics of the patients

	New Smear positive	New Smear Negative	Statistical test value	p value
Mean age	44.4± 18.5	39.6± 17.0	7.23*	0.000000 #
Total cases	1458	1412		
Male	1062 (72.8)	946 (67.0)	3.37**	0.000745 #
Female	396 (27.2)	466 (33.0)	3.37**	0.000745 #

*student's t test
** z test for proportion
# Statistically significant

Table 2: Comparison of the outcomes of new smear positive and smear negative cases

		New Smear positive	Percentage	New Smear Negative	Percentage	p value
Favourable outcome	Cured	1259	86.35	0		
	Treatment completed	25	1.71	1188		
	Total	1284	88.06	1188	84.13	0.002777 #
Unfavourable Outcome	Death	54	03.70	96	06.80	0.000272 #
	Failure	36	02.47	21	01.49	0.079940
	Default	61	04.18	85	06.02	0.031326 #
	Transferred Out	23	01.58	22	01.56	0.913690
	Total	174	11.93	224	15.86	0.002777 #

statistically significant

significantly higher in new smear positive group (Table 2).

DISCUSSION

Sputum negative pulmonary tuberculosis constitutes about 50% of all new cases of pulmonary tuberculosis. Although the relative transmission rate of smear negative tuberculosis is lower than that of smear positive cases, it is still responsible for 17% of tuberculosis transmission[3].

In the RNTCP, the diagnosis of smear negative pulmonary tuberculosis rests upon clinical symptoms and the chest X-ray. The RNTCP recommends the screening of patients with symptoms of TB. The symptoms that are used for the screening of these patients are a productive cough for three weeks or more with or without haemoptysis, fever, chest pain, weight loss or night sweats who present on their own initiative at health facilities[4].

However, these symptoms, although seen in smear positive patients, are not classically present in smear negative PTB. So much so, that the presence of expectoration is considered a negative predictive factor for sputum negative tuberculosis and an alternative diagnosis, for example, bacterial pneumonia, chronic bronchitis or bronchiectasis, is considered[5]. The chest X-ray also, has been conclusively proved to be of little value in the diagnosis of tuberculosis[6,7], especially in patients with sputum negative pulmonary tuberculosis[8].

Patients with sputum negative tuberculosis have a smaller mycobacterial burden and therefore their clinical and radiological manifestations are different from those with smear positive PTB[5,9] .

The present study shows that the incidence of favourable outcome in cases of smear negative tuberculosis is only 84%, lower than that targeted by the WHO. Sputum positive tuberculosis on the other hand carries a favourable outcome of 88%.

It is generally believed that before the advent of the HIV epidemic smear negative pulmonary tuberculosis was associated with a good prognosis[10]. Since then, there has been an increase in the adverse outcomes in areas with high incidence of HIV. The primary reason for this is an over diagnosis of smear negative pulmonary tuberculosis on the chest radiographs in the presence of other opportunistic HIV related infections[9]. In the reporting unit, although the exact incidence of HIV infection is not known, it is likely to be low since the incidence of HIV in STD clinics in West Bengal is 0.88[10]. The increased incidence of adverse outcome, therefore, is due to factors other than HIV.

The decreased incidence of favourable outcome in smear negative cases was seen to be due to the differences in the incidence of defaults and deaths among these groups.

In their study in Pakistan, Kamran Siddiqui et al[11] found that 6.1% patients declared AFB smear negative from a tuberculosis programme laboratory were AFB smear positive when re-examined in a reference laboratory. Increased incidence of failure among smear negative pulmonary tuberculosis could be the result of improper categorization resulting in the prescription of inappropriate drugs. However, further studies to identify causes of failure must be undertaken.

Default is significantly higher in patients with sputum negative PTB. Several socio-economic, demographic, drug and occupational factors, that have been found to be associated with an increased incidence of default from anti-tubercular therapy[12], are likely to be present equally in both the groups since they belong to the same population. During the initial years of the implementation of the RNTCP, more stress was given to the identification and cure of sputum positive patients. This lack of attention of the tuberculosis programme workers towards the sputum negative group resulted in more defaults[13]. This study includes patients since 1999, when the DOTS programme was initially started in the area.

The present study observed that smear negative patients had significantly higher deaths. Patients with sputum negative PTB, seen in this study, have a significantly lower mean age than the other group and hence a decreased physiological chance of death. The other important reasons for the increased incidence of death in smear negative PTB can be i) prevalence of HIV, ii) delay in the diagnosis of patients in this group and iii) inaccurate diagnosis under operational conditions.

In areas with low incidence of HIV, delay in diagnosis is an important cause of increased incidence of death in patients with smear negative pulmonary tuberculosis. This delay from the appearance of symptoms to the time of diagnosis of the disease can be divided into two intervals. Patient delay defined as the time interval from the appearance of the major pulmonary symptoms of the disease until the first visit to the medical facility and health service delay defined as the time interval from the first consultation until the date of diagnosis[14]. Studies have shown that the mean time interval in the delay in seeking medical attention that is the patient delay is higher in patients with smear negative pulmonary tuberculosis[15]. This suggests that these patients seem to stay at home until they notice an alarming symptom or are grossly incapacitated by the disease.

Radiological facilities under programme conditions are available at the higher centres of treatment, which in most cases are located far from the patients' residence. Distance from the nearby health care facility has been shown to be linked to the increase in the delay in seeking medical treatment. In the study population access to radiological facilities and often economic constraints of radiological investigations result in the delay in diagnosis of smear negative tuberculosis.

This delay in the diagnosis causes patients to be more seriously ill at the time of presentation. In the current study approximately 25% of patients were seriously ill at the time of diagnosis. Analysis of all deaths in the smear negative group shows that about 35% of patients who died during therapy were seriously ill at the time of presentation.

Diagnosis of smear negative tuberculosis should take at least 15 days under programme conditions, which should be the optimum health service delay. However, in their study in Ethiopia, Demissie M. et al[15] found that 85% of patients were diagnosed in less than 15 days which indicates that the correct diagnostic procedure for the diagnosis of these cases is not being followed. This will result in several false positive cases and an over-diagnosis of smear negative pulmonary tuberculosis.

Bacterial pneumonias are the commonest cause of misdiagnosis on the radiographs and may occur in 14.0 to 41.2 % of cases[15-19]. Other lung diseases mimicking tuberculosis are interstitial pneumonitis, carcinoma, lymphoma, chronic

obstructive pulmonary diseases, Interstitial lung diseases, occupational lung diseases like silicosis, etc.

The use of clear cut radiological criteria can help in better detection of pulmonary tuberculosis. In their study in Kenya, van Cleeff MR et al[8] has shown that the introduction of a four point scoring system, improved the diagnosis of smear negative cases by reducing over-diagnosis up to 67%, while only 8% fewer culture positive cases would start immediate treatment. In the absence of clear cut radiological criteria under the RNTCP, difficulties in the interpretation of chest radiographs are common.

The training given to the medical students in the undergraduate programmes or the medical officers under the RNTCP is not adequate in the interpretation of radiological reports, especially in the detection of smear negative tuberculosis. Moreover, although the interpretation of the chest radiograph is more difficult than a sputum smear result, quality control is hardly practised.

Follow-up of sputum smear negative patients, at the end of the intensive phase, under the RNTCP is by sputum microscopy. Radiological follow-up at the end of intensive phase will give an opportunity for the re-assessment of the diagnosis, and plan the use of ancillary investigations like sputum culture in patients without significant radiological improvement at the end of the intensive phase.

The limitations of our paper are that we have not done HIV, X-ray, diabetes status, and duration of symptoms analysis as it is not available under programme conditions. A more detailed study incorporating all these would be helpful.

To conclude, sputum negative tuberculosis constitutes almost half the cases of pulmonary tuberculosis. We believe that delayed presentation, delay in the diagnosis and improper diagnosis under field conditions in smear negative cases are responsible for the decreased incidence of favourable outcome in low HIV areas. We recommend a more intense training of medical students during their undergraduate courses and health service doctors involved in the RNTCP, formulation of clear cut radiological guidelines for the detection of pulmonary tuberculosis, and the establishment of quality control in the interpretation of chest X rays in the diagnosis of smear negative tuberculosis. We also recommend the routine use of chest radiographs along with sputum smear examination at the end of the intensive phase for follow up of smear negative cases.

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Indian J Tuberc 2009; 56:151-153

STATUS REPORT ON RNTCP*

The RNTCP has continued to achieve the twin objectives of NSP case detection and treatment success rate at the national level during the first quarter, 2009.

RNTCP performance during first quarter 2009

During the quarter, over 1.79 million suspects were examined, 228,754 sputum positive cases were diagnosed, and a total of 372,619 TB cases (which include sputum smear negative and extra-pulmonary TB patients) were registered for treatment. The treatment success rate amongst the new smear positive PTB cases registered in the first quarter 2008 is 87% and the sputum conversion rate of patients registered during fourth quarter 2008 is 90%.

Major Activities during the quarter

TB-HIV collaborative activities

All the nine states implementing Intensified TB/HIV Package services have started reporting on the status of routine referral of all TB patients for HIV counselling and testing. With the expansion of Intensified TB/HIV package of services to Delhi and Gujarat during 2009, the number of states implementing this package has increased to 11 states. It was decided to expand the package in a phased manner, to cover the entire nation by 2012.

The National Laboratory Committee meeting

The 16th meeting of National Lab Committee was held in March, 2009. The laboratory scale-up plan for the diagnosis and management of MDR-TB which includes establishment of 43 solid culture and LPA units and 33 liquid culture units was endorsed by the committee. The laboratory performance indicator and generic standard operating procedures for C&DST labs were also approved by the committee. Six Institutions under the ICMR, Government of India were identified for participating in the DOTS-plus diagnosis services, after undergoing accreditation from the Tuberculosis Research Centre, Chennai.

Progress in accreditation of Intermediate Reference Laboratories (IRL)

The IRLs of Tamil Nadu and Rajasthan were accredited under RNTCP during the quarter and it is currently undertaking culture and DST services for the MDR TB suspects from the state. Another six IRLs (Haryana, West Bengal, Uttarakhand, Chattisgarh, Jharkhand and Orissa), three medical college labs and three private labs are under the accreditation process and are expected to be accredited in 2009.

Progress in DOTS-Plus services for MDR-TB cases

During this quarter, DOTS Plus treatment services have been initiated in Tamil Nadu, thus bringing the total number of states implementing DOTS Plus to eight. By the end of this quarter, over 400 MDR-TB cases were on RNTCP Category IV treatment in the country. The state of Rajasthan has started the identification of MDR suspects and will begin the treatment services shortly. The states of Orissa and Uttar Pradesh are in advance stage of preparation and are expected to roll out the services shortly.

Progress in the involvement of NGOs and PPs

The involvement of health institutions under the Catholic Bishop's Conference of India (CBCI) began during the third quarter 2008. One remaining state level workshop was conducted, thus completing this activity in all 11 identified states. In addition, 59 new NGO-PP Memoranda of Understanding were signed between the state/ districts and the Catholic Health Facilities.

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

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

State	Population (in lakh) covered by RNTCP[1]	Suspects examined per lakh population	No of Smear positive patients diagnosed[2]	Total patients registered for treatment[3]	Annualized total case detection rate	New smear positive patients registered for treatment	Annualized new smear positive case detection rate (%)		No of new smear negative cases registered for treatment	No of new EP cases registered for treament	No. of smear positive retreatment cases registered for treatment	3 month conversion rate of new smear positive patients	Cure rate of new smear positive patients	Success rate of new smear positive patients
Andaman & Nicobar	4	236	115	214	204	94	90	119%	56	45	14	93%	83%	83%
Andhra Pradesh	830	160	19034	28537	137	12245	59	79%	7747	3245	3898	92%	86%	88%
Arunachal Pradesh	12	203	273	568	187	198	65	87%	141	93	74	91%	82%	86%
Assam	304	112	5107	9185	121	3824	50	67%	2651	1194	817	90%	86%	88%
Bihar	953	75	9769	16891	71	7409	31	41%	5326	1169	1449	90%	82%	88%
Chandigarh	11	352	480	569	209	204	75	79%	97	169	56	94%	84%	85%
Chhatisgarh	240	115	3199	6914	115	2553	42	53%	2826	827	398	88%	82%	87%
D & N Haveli	3	174	74	89	131	33	49	61%	20	16	12	92%	84%	84%
Daman & Diu	2	396	38	86	178	32	66	83%	26	9	10	93%	50%	61%
Delhi	176	244	6660	12967	295	3807	87	91%	2156	4096	1718	91%	86%	87%
Goa	17	175	293	468	112	145	35	43%	112	130	50	90%	77%	80%
Gujarat	572	180	15448	19971	140	8722	61	76%	2798	2684	4138	92%	86%	87%
Haryana	241	162	6034	8768	145	3223	53	56%	1683	1528	1764	91%	84%	84%
Himachal Pradesh	66	223	2090	3412	206	1306	79	83%	560	781	533	93%	87%	89%
Jammu & Kashmir	128	175	1974	3258	102	1414	44	47%	543	778	410	92%	89%	91%
Jharkhand	304	110	5082	9202	121	4055	53	71%	2984	756	650	91%	83%	89%
Karnataka	580	181	10274	16545	114	6327	44	58%	3739	3261	2153	87%	78%	80%
Kerala	346	225	4062	6660	77	2943	34	68%	1386	1491	624	83%	81%	83%
Lakshadweep	1	61	3	3	17	2	11	15%	1	0	0	100%	100%	100%
Madhya Pradesh	705	105	11503	20295	115	7272	41	52%	6537	2532	2680	89%	83%	87%
Maharashtra	1083	146	19409	35801	132	13110	48	61%	8975	6757	3867	90%	82%	84%
Manipur	27	115	303	839	126	208	31	42%	285	169	65	85%	86%	87%
Meghalaya	26	154	548	988	154	359	56	75%	162	249	128	85%	79%	80%
Mizoram	10	236	208	622	251	150	60	81%	196	185	45	92%	93%	95%
Nagaland	22	150	372	890	161	314	57	76%	241	154	102	92%	89%	90%
Orissa	403	138	7396	12703	126	5519	55	64%	3175	2401	955	88%	82%	86%
Puducherry	11	296	459	285	104	134	49	65%	49	64	35	88%	86%	86%
Punjab	269	162	6092	9300	138	3879	58	61%	1603	1818	1576	90%	84%	87%
Rajasthan	657	146	17042	27062	165	9733	59	74%	7711	3421	4998	91%	87%	89%
Sikkim	6	320	210	440	293	119	79	106%	87	125	72	93%	83%	83%
Tamil Nadu	669	218	10934	20738	124	7979	48	64%	5762	4249	2003	90%	85%	86%
Tripura	36	181	416	709	80	363	41	54%	147	128	51	91%	91%	91%
Uttar Pradesh	1944	151	44506	68611	141	31373	65	68%	17783	7949	8786	91%	85%	88%
Uttarakhand	96	170	2215	3181	132	1190	49	52%	686	517	569	88%	80%	84%
West Bengal	889	169	17119	26066	117	12188	55	73%	4937	4237	2967	90%	84%	85%
Grand Total	**11641**	**152**	**228741**	**372837**	**128**	**152426**	**52**	**70%**	**93188**	**57227**	**47667**	**90%**	**84%**	**87%**

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

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

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

Under the Global Fund round, six RNTCP IMA PPM projects and 118 CMEs were held and 4888 PPs were sensitized during this quarter. 47 DTPs were held and 1005 PPs were trained.

Progress under NRHM and GFATM

The appraisal of NRHM PIPs for all states and UTs was done during February-March'09 by the NRHM Division and Programme Officers.

Under GFATM, the first Rolling Continuation Channel (RCC) TB Grant has been approved by the GF Board. The Project Implementation Plan (PIP) for six years has been prepared and submitted to the Expenditure Finance Committee (EFC) for approval.

Progress in Advocacy, Communication and Social Mobilization

World TB Day was observed on 24th March, 2009 across the country in all the states and districts. On this day, intensive efforts were made with all the stake holders to highlight the burden of TB and the progress made by RNTCP for reducing this burden. Central TB Division released TB-India, 2009, the annual report on the progress made under RNTCP.

Case Report

STERNAL TUBERCULOUS OSTEOMYELITIS PRESENTING AS A PULSATILE SWELLING

Hari Kishan Boorugu*, Anugrah Chrispal* and Elsa Mary Thomas*

(Received on 18.11.2008; Accepted after revision on 23.4.2009)

Summary*:** Primary sternal tuberculous osteomyelitis is a rare form of tuberculous osteomyelitis. We report a case of a young adult with primary tuberculous osteomyelitis of the sternum who presented with a pulsatile anterior chest wall swelling. Computed Tomography of the thorax revealed a hypodense lytic lesion in the body of the sternum that had eroded into the anterior mediastinum where it lay in close contact with the right ventricle, resulting in the clinically evident transmitted pulsations. Among the protean manifestations of tuberculosis this case illustrates a unique presentation as a pulsatile chest wall mass. ***[Indian J Tuberc 2009; 56:154-156]

Key Words: Sternal osteomyelitis, Tuberculosis, Pulsatile sternal swelling

INTRODUCTION

Sternal osteomyelitis may be of primary and secondary forms. The more commonly seen secondary sternal osteomyelitis is usually a complication of sternotomy following procedures as coronary artery bypass grafts, and may be caused by atypical mycobacteria and other pyogenic bacteria[1]. Sternal osteomyelitis per se is a rare disease accounting for 0.3 to 1.8% of all cases of osteomyelitis[2]. Isolated tuberculous sternal osteomyelitis comprises less than 1% of musculoskeletal tuberculosis[3]. Two studies from India have reported 14 and 19 cases of sternal tuberculosis between 2000 and 2006 highlighting the relative rarity of this condition[4,5]. Sternal tuberculosis may mimic a variety of conditions of the sternum including pyogenic osteomyelitis, actinomycosis, fungal infections, sarcoidosis and malignancies[6]. Tuberculous sternal osteomyelitis usually occurs in young adults who present with insidious onset pain, discomfort and swelling in the sternal region[4]. We present a young adult with a painless pulsatile sternal swelling which was found to be of tuberculous origin.

CASE HISTORY

A 28-year old man from Tamil Nadu, presented with a gradually increasing, pulsatile, painless swelling over the mid sternal region for three months. During this period, he had low grade, intermittent fever, anorexia and significant weight loss. He denied a history of high risk behaviour or blood transfusions in the past. On examination he was emaciated and pale. He had no peripheral lymphadenopathy. He had a 3X3 centimetre, well defined, non-tender, pulsatile swelling in the mid sternal region which was smooth and fluctuant with no evidence of inflammation. These pulsations appeared to be 'transmitted' and not 'expansile' on clinical examination. Per abdominal examination revealed an ill-defined mass in the left hypochondrium which moved well with respiration, which subsequently was found to be a lymph node mass on abdominal ultrasonography.

Investigations revealed a dimorphic anaemia (Haemoglobin 8.4 gram %) and erythrocyte sedimentation rate (ESR) of 140 millimetres at one hour. Liver function tests revealed hypoalbuminemia

* Assistant Professor, Departments of Medicine 2 and Radiology, Christian Medical College and Hospital, Vellore.
Correspondence: Dr. Hari Kishan Boorugu, Assistant Professor, Department of Mexicine 2, Christian Medical College and Hospital, Vellore-632 004, (Tamil Nadu). Fax number: 0416 2232035; Phone numbers: Landline -04162282031; Mobile: 09994068911; E-mail: drharikishan@gmail.com

(Serum Albumin – 2.6 gram %). HIV ELISA was negative. Chest radiography showed significant mediastinal widening and there were no infiltrates in the lung fields to suggest parenchymal involvement. Abdominal Ultrasonography revealed multiple enlarged mesenteric and omental lymph nodes.

A Computerized Tomogram (CT) of the chest revealed a hypodense lytic lesion eroding the body of the sternum, with chest wall and intra-thoracic extension, abutting the right ventricular outflow tract (Figure), suggestive of osteomyelitis of the sternum with cold abscess formation. There were multiple enlarged, necrotic, matted mediastinal and hilar lymph nodes. Subcarinal nodes had eroded into the oesophagus forming a fistulous tract. There was no parenchymal involvement evident on CT. White, cheesy material was aspirated from the chest wall swelling, a smear of which revealed numerous acid fast bacilli on Ziehl-Neelsen stain. Aspirates from intra abdominal lymph nodes and the chest wall swelling subsequently grew *Mycobacterium tuberculosis* on culture. The patient was thus diagnosed to have disseminated tuberculosis with sternal tuberculous osteomyelitis and cold abscess. He was initiated on anti-tuberculous therapy with Rifampicin (450 mg once daily), Isoniazid (300 mg once daily with Pyridoxine), Ethambutol (800 mg once daily) and Pyrazinamide (1250 mg daily). He was advised to take all four drugs for the initial two months and then switch to continuation phase with Rifampicin and Isoniazid for the next 10 months. He was advised surgical repair of the sternal defect at a subsequent date. He, however, was lost to follow up.

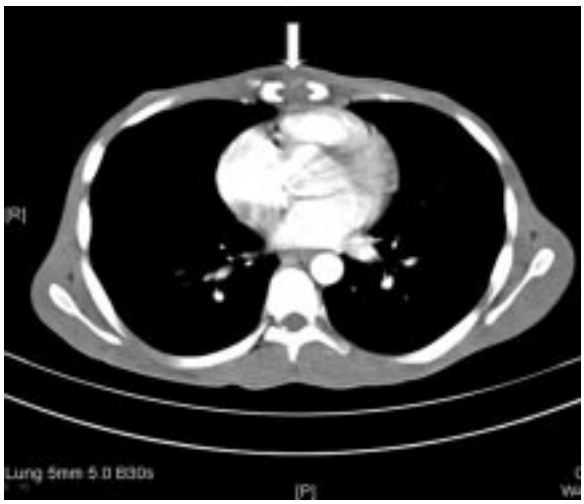

Figure: Axial contrast-enhanced CT scan of the thorax showing a hypodense lytic lesion in the body of the sternum, with cortical breach, extending anteriorly into the chest wall and posteriorly into the anterior mediastinum, abutting the right ventricular outflow tract

DISCUSSION

This patient had a sternal tuberculous osteomyelitis with a cold abscess that had eroded into the thorax and was pulsatile by virtue of its proximity to the anteriorly placed right ventricle and its outflow tract. Tuberculous sternal osteomyelitis is a rare presentation of tuberculosis. This case, however, was unique due to its presentation as a pulsatile sternal swelling which, to the best of our knowledge, has not been described previously.

Usually, tuberculous sternal osteomyelitis is caused by reactivation of latent foci of primary tuberculosis due to lymphatic or haematogenous dissemination. Occasionally, it may be due to direct extension from contiguous mediastinal lymph nodes[3]. Tuberculous sternal osteomyelitis tends to involve the body of the sternum more often than the manubrium. The indolent nature of the swelling helps distinguish tuberculous sternal osteomyelitis from other pyogenic osteomyelitis caused by Staphylococcus aureus and Gram Negative Bacilli. On occasion sternal tuberculous osteomyelitis has presented with sternal fracture and draining sinuses[7]. Complications of sternal osteomyelitis include secondary infection, fracture, fistula formation, compression of trachea, compression and erosion of underlying blood vessels, extension of the tubercular abscess into surrounding tissues including the mediastinum, pleural cavity and subcutaneous planes[3].

Diagnosis rests on histopathological and microbiological confirmation from the sternal tissue

or pus from the cold abscess[6]. Radiographic abnormalities tend to lag behind the clinical manifestations, and may mimic other benign and malignant conditions[8]. Computed Tomography of the chest is excellent for delineation of extent of sternal destruction as well as soft tissue extension. Magnetic Resonance Imaging (MRI) is probably superior for detecting early marrow oedema and soft tissue involvement[3].

Treatment is based on anti-tuberculous chemotherapy in combination with drainage and debridement of necrotic material, although a number of cases have been treated with anti-tuberculous agents alone. There is no consensus on the role of adjuvant surgical interventions in patients with sternal tuberculosis. Debridement is the mainstay of surgical treatment along with sternal defect closure by flap repair (pectoralis major, latissimus dorsi, rectus abdominis or omental flap closure) with or without chest wall reconstruction[4]. Vacuum assisted closure has been tried successfully[7].

Presentation of a pulsatile chest wall mass should alert the clinician to possible differential diagnoses including vascular malformations, aneurysms and vascular metastatic lesions. Among the protean manifestations of tuberculosis this case illustrates a unique presentation as a pulsatile chest wall mass.

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Case Report

PRIMARY MULTI-DRUG RESISTANT TUBERCULAR LYMPHADENITIS IN AN HIV INFECTED PATIENT

Jagdish Rawat[1], Girish Sindhwani[1] and Ruchi Dua[2]

(Received on 2.1.2009; Accepted after revision on 26.5.2009)

Summary:: Cervical lymphadenitis is a common extra-pulmonary manifestation of tuberculosis in HIV patient; nevertheless, it seems that the primary Multi Drug Resistant (MDR) involving extra-pulmonary site is uncommon. We report a case of tubercular lymphadenitis by multi-drug resistant strain of *Mycobacterium tuberculosis* in an HIV seropositive male, which has not been reported so far in literature. ***[Indian J Tuberc 2009; 56:157-159]***

Key Words: Tuberculosis, Lymphadenitis, HIV Infection

INTRODUCTION

HIV infection is rising and is emerging as the most important risk factor for the developing tuberculosis in India. About 2.4 million people are infected with HIV, about half of which are co-infected with *M. tuberculosis*[1].

Primary MDR extra-pulmonary tuberculosis is an uncommon form of the disease, but it seems that due to increasing prevalence of drug resistant tuberculosis around the world, the number of cases of primary MDR tuberculosis presenting at extra-pulmonary sites is going to rise especially in patients with HIV infection. In this report, we present a 32 years' old, HIV positive male with primary MDR lymphadenitis of cervical and axillary region.

CASE REPORT

A 32-year, non-smoker male was admitted to our hospital in July 2006 for investigation of enlarged left superaclavicular, lower cervical and left axillary lymph node. He had a positive history of non-specific fever, loss of appetite and weight but history of dyspnoea, chest pain, cough, sputum production and night sweats was absent. No history of any close contact with a recognized TB patient could be elicited. He was a tea stall keeper by occupation.

On physical examination, a mobile, non-tender, non-matted lymphnode of 2 x 3cm size was observed in left lower cervical region along with two non-tender, matted lymph nodes of size 1.5 x 2.5 cm in left supraclavicular and one non-tender, non-matted lymph node of size 2.5x 2.5 cm in left axilla (Fig. 1). No palpable lymph nodes were found in other sites. The physical examination of other

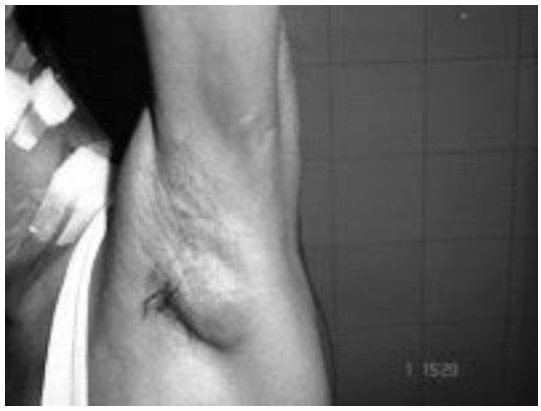

Figure 1: Lymph node swelling of size 2.5 x 2.5 cm in left axilla.

1. Assistant Professor 2. Senior Resident
Department of Pulmonary Medicine, Himalayan Institute of Medical Sciences, Dehradrun.
Correspondence: Dr. Girish Sindhwani, Assistant Professor, Department of Pulmonary Medicine, Himalayan Institute of Medical Sciences, Dehradun (Uttarakhand); Phone: +911352471362, 9411718286; Fax: +911352471317; e-mail: girish_sndhwani@rediffmail.com

organ systems was normal. The haematological and biochemical parameters were normal and a tuberculosis skin test for *M.tuberculosis* showed 7mm.induration. X-ray chest PA view was unremarkable. Serum samples were positive for antibodies against HIV using HIV comb immunoassay (J. Mitra & Co. Ltd.), followed by HIV microlisa kit (J. Mitra & co. Ltd.). Patient gave history of blood transfusion in past, that might be the possible source of infection. CD4+ cell count was 139/MicroL. Ultrasound examination of whole abdomen showed retroperitoneal lymphadenopathy and mild hepatomegaly. FNAC of left cervical and supraclavicular lymph node showed chronic granulomatous inflammation consistent with tuberculosis. The acid fast bacilli of the specimen were negative. For confirmation of diagnosis, pus was also sent for culture and sensitivity for tuberculosis by Lowenstein – Jensen method at an intermediate reference laboratory (New Delhi TB Centre, NRL).

Patient was put on anti-tubercular treatment (ATT) under DOTS, CAT-I. Pus aspirated from the cold abscess in the neck grew *M.tuberculosis* complex, resistant to Streptomycin, Rifampicin and Isoniazid. . During this three month period till the availability of culture and sensitivity report, there was no significant improvement in lymph node size.

His ATT regimen was modified according to sensitivity report to Kanamycin, Ethionamide, Ethambutol, Pyrazinamide and Levofloxacin according to body weight. He was also put on anti-retroviral therapy (ART) (Lamivudine+stavudine+Efavirenz). Kanamycin was stopped after three months and remaining drugs continued (3 months of intensive phase and 15 months of continuation phase). At the end of eighteen months, complete resolution of lymph nodes occurred with some residual scarring (Figs. 2 & 3). ATT was stopped with continuation of ART. At the end of treatment, CD4+ cell count was 251/ microL.

DISCUSSION

Drug resistant tuberculosis has become a major public health problem since early 1990[2]. The prevalence of primary multi drug resistant tuberculosis in India was estimated around 2-3%; however, this prevalence is 15-50% among previously treated cases.

In HIV seronegative and immuno-competent patients, pulmonary tuberculosis is the commonest mode of presentation, while EPTB accounts for only 20% of cases but in HIV positive patients it accounts for nearly 50-55 %[3]. Among extra-pulmonary tuberculosis, cervical lymphadenitis is the commonest presentation; nevertheless, it seems that the primary MDR of EPTB is an uncommon form of the disease in patients of HIV infection. The reported patient is the first HIV infected patient with primary MDR tubercular lymphadenopathy in India to the best of our knowledge.

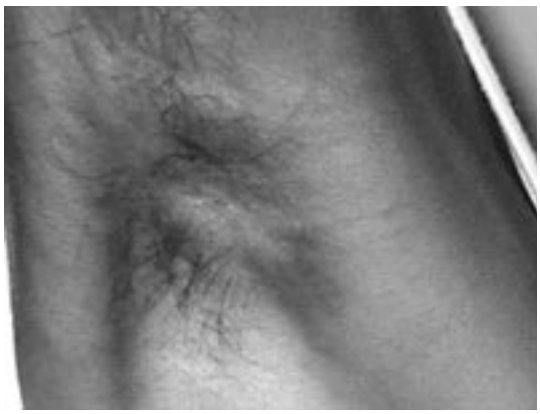

Figure 2: Axillary lymph node disappeared after treatment.

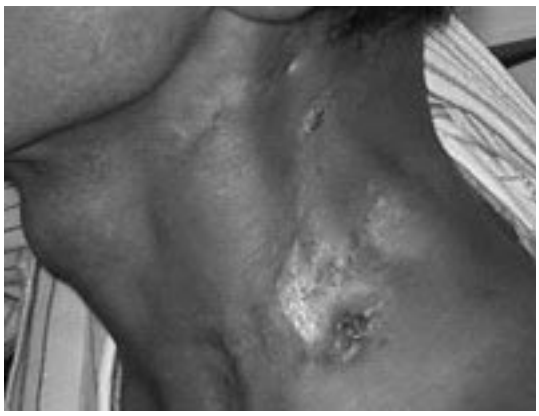

Figure 3: Cervical lymph node healed by scarring.

This case also demonstrates the possibility, that even if the absolute number of CD4+cell count appeared to be enough to prevent opportunistic infection, there is still always a chance of developing opportunistic infection.

Our patient tolerated second line ATT and antiretroviral therapy well without significant complications which has been similarly reported by others[4].

With increasing prevalence of drug resistant TB cases around the world, such manifestations of disease, including the primary MDR tuberculosis at extra-pulmonary sites are expected to be higher than before, which brings emphasis on rapid and reliable diagnosis and increasing awareness of physicians to such presentations.

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Case Report

TUBERCULOSIS OF THE MIDDLE EAR WITH POST AURICULAR ABSCESS

Manoj Arya, Ramakant Dixit, A.R. Paramez, Sidharth Sharma and Dilip Singh Rathore

(Received on 1.5.2009; Accepted after revision on 18.6.2009)

Summary: A case of tuberculous otitis media with post auricular abscess is being described in a 14 year old female patient in view of its rare occurrence. The diagnosis was made on demonstration of acid fast bacilli (AFB) in the ear discharge and characteristic cytological features of post auricular abscess aspirate. ***[Indian J Tuberc 2009; 56:160-163]***

Key words: Tuberculosis, Middle ear, Post auricular abscess

INTRODUCTION

Tuberculosis of the middle ear is a rare clinical entity that is usually seen in association with or secondary to pulmonary tuberculosis[1]. The true incidence of this condition is difficult to assess, however in children, tuberculous otitis media (TOM) has been accounted for 0.04% of' all cases of chronic suppurative otitis media[2]. These patients usually present with painless otorrhoea that fails to respond to the usual antimicrobial treatment in patients with evidence of tuberculosis elsewhere in the body[3].

This report describes an apparently healthy 14-year-old female child having painless ear discharge with hearing impairment and post auricular abscess on left side due to tuberculosis.

CASE REPORT

A 14-year-old female child presented with painless discharge from left ear for four months with decreased hearing from the same and swelling behind the affected ear for the last two months. She had no history of fever or any other complaint. There was no history of previous illness and no history of tuberculosis in the family. She was treated by several courses of broad spectrum antibiotics both systemic and local.

On physical examination, she was a healthy female child with body mass index 25. There was no anaemia, lymphadenopathy or any other abnormality on systemic examination. On otoscopy, there was thick pus seen in left external auditory canal which after removal showed three perforations in the tympanic membrane. There was a soft fluctuant, slightly tender swelling at left post auricular area measuring 4 x 5 cm with crusty scaling external surface (Figure 1). Local examination of right ear, nose, oral cavity and pharynx were normal.

Her laboratory investigations revealed haemoglobin 13 gm%, total leucocyte count 7900

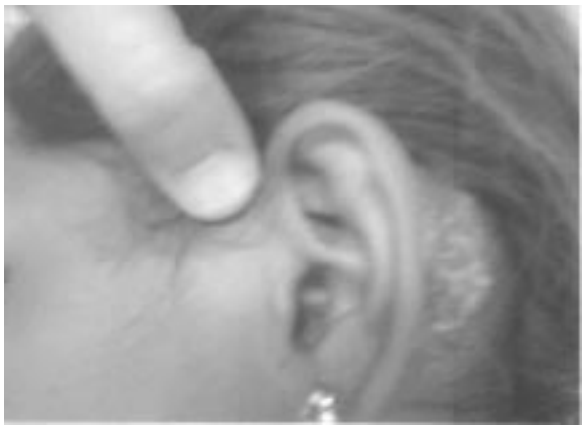

Figure 1: Photograph of patient showing thick purulent ear discharge with post auricular abscess.

Department of Respiratory Medicine & Tuberculosis, JLN Medical College, Ajmer, (Rajasthan)
Correspondence: Dr. Ramakant Dixit, 381/26, Ramganj, Ajmer-305 001, Rajasthan. Tel: 91-145-2691542.
E-mail: dr.ramakantdixit@gmail.com

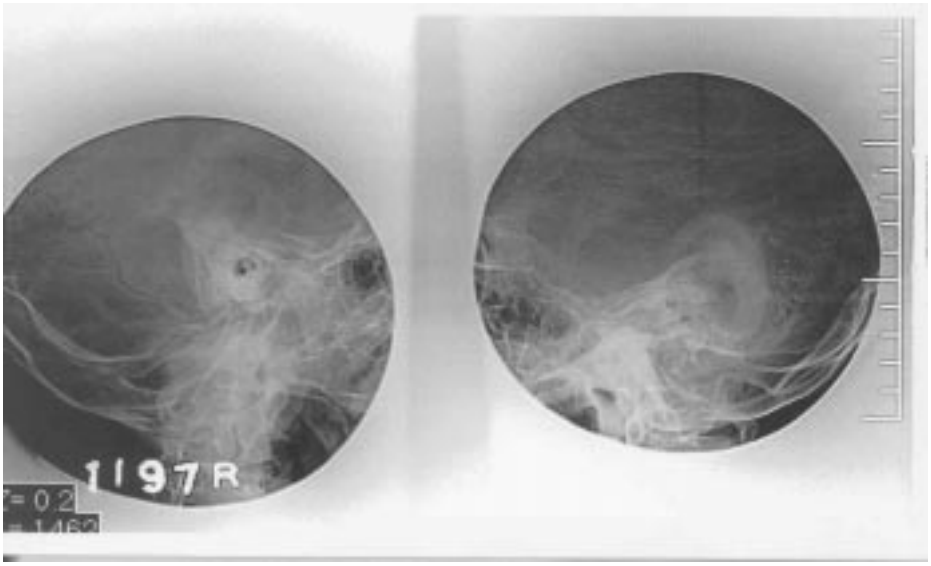

Figure 2: Digital X-ray of mastoid region (Law's lateral oblique view) showing bilateral absence of mastoid air cells with radio opaque densities seen more on left side suggestive of chronic mastoiditis.

cells/mm^3 (polymorphs 71%, lymphocytes 25%, eosinophils 3 %), ESR 40 mm in 1st hour. Mantoux test revealed an induration of 14 mm. Other investigations such as liver function test, renal function test, HIV, fasting blood sugar, etc., were within normal limits.

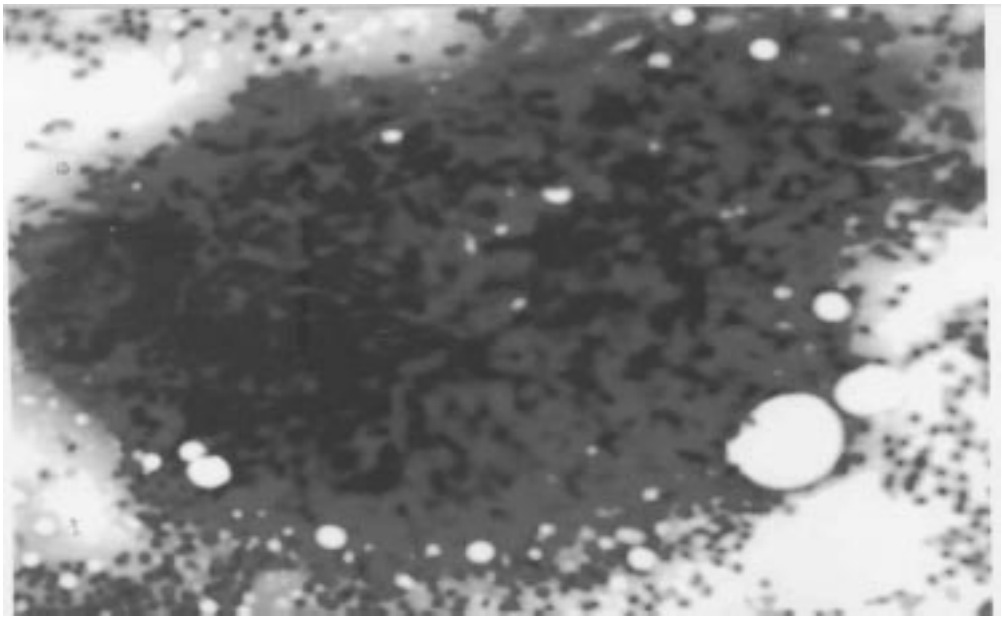

Figure 3: Photomicrograph of post auricular abscess cytology showing clusters of epithelioid cells, caseous necrotic background and lymphoid cells (Giemsa 400)

Digital X-ray chest was normal. Digital X-ray of mastoids region (Law's lateral oblique view) showed bilateral absence of mastoid air cells with radio opaque densities seen more on left side suggestive of chronic mastoiditis (Figure 2).

Direct smear from ear discharge revealed numerous AFB on ZN staining (3+). Post auricular abscess was drained anti-gravity, under local anaesthesia and about 10 ml of thick pus with blood mixed with granulation tissue was removed. Cytological and histological examination of drained pus was consistent with tuberculosis i.e. showing collection of epithelioid cells, multinucleated giant cells and lymphocytes in caseous necrotic background (Figure 3). Child was managed by anti-tuberculosis chemotherapy as category III treatment under RNTCP, with local dressing of post auricular lesion. There was good clinical response following above therapy with complete cessation of ear discharge at the end of two weeks. There was no recurrence of post auricular abscess at the end of two months. The child was referred to ENT specialist for surgical opinion where she was advised to report after completion of medical treatment. However, the child did not come for follow-up thereafter.

DISCUSSION

The true incidence of TOM is difficult to assess, as the large reported series have been selected from hospitalized sub-groups with established tuberculosis[4-5]. It is known to occur in all age groups but is more common in children. Primary tuberculosis of ear has rarely been reported and disease is usually secondary to infection in lung, larynx, pharynx and nose[6]. In the present case, the tuberculous involvement of middle ear seems primary in view of lack of evidence of tuberculosis infection at other body sites.

The route of spread of tuberculosis to middle ear has been argued for many years; however the most accepted theory support entry of organism via pharyngotympanic tube[4]. Infection can also reach the middle ear via external auditory canal or by hematogenous spread. Very rarely the middle ear cavity may also be involved in congenital tuberculosis[7].

Generally, there is unilateral involvement of ear in tuberculosis with characteristic presentation of painless otorrhoea not responding to antimicrobial treatment in patients with tuberculosis at other body site. There is variable degree of hearing impairment that is out of proportion to the apparent degree of development of disease seen on otoscopy. It can be conductive deafness, sensory neural or mixed. There is multiple tympanic membrane perforation, abundant granular tissue, bone necrosis and preauricular lymphadenopathy. Periauricular lymphadenopathy and fistula are infrequent findings. Late complications include facial paralysis, labyrinthitis, post auricular fistulae, subperiosteal abscess petrous apicitis and intra cranial extension of infection[8]. In the present case, the child presented with painless ear discharge, mild deafness, multiple tympanic membrane perforation and local extension of lesion to cause post auricular abscess without any neurological or serious complications.

The differential diagnosis of TOM includes fungal infection, wegner's granulomatosis, midline granulomas, sarcoidosis, syphilis, necrotizing otitis extema, atypical mycobacterial infection, lymphoma, histiocytosis- X and cholesteatoma[9]. These conditions can be excluded clinically by the presence of ear ache, and the type and consistency of the discharge and by further diagnostic laboratory workup.

The role of X-ray of mastoid and CT scan is limited in view of non-specific finding but together with clinical and other laboratory tests, it helps in strengthening the diagnosis in suspected cases. The diagnosis of TOM is based on the demonstration of AFB within the granuloma in biopsy tissue, ear discharge or middle ear aspirate with or without culture for *Mycobacterium tuberculosis*. The positivity of the AFB in ear discharge varies from 5-35% and may be improved to 50% after repeated examinations[10]. If facility is available, ear discharge may be subjected to polymerase chain reaction (PCR) test for *Mycobacterium tuberculosis*.

The treatment of choice for TOM is anti-tuberculosis chemotherapy using currently available combinations. Surgery may be required in some cases to remove sequestra and to improve drainage. In view of effective anti-tuberculosis drugs available, the role of surgery in today's practice is limited and should be reserved for decompression of facial nerve, for removal of necrotic material which might provide a nidus for the organism to remain out of reach of anti-tuberculosis drugs. When surgery is combined with adequate chemotherapy, there is better healing with good prognosis. When anti-tuberculosis therapy is started early, the mortality is negligible[11].

In conclusion, TOM is a rare condition which if untreated can damage middle ear and surrounding structures. It should always be considered in the differential diagnosis of chronic ear discharge not responding to usual antimicrobial therapy. A high level of clinical suspicion is needed for early diagnosis and anti-tuberculosis therapy should be started as soon as possible to prevent possible complications.

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ABSTRACTS

Predictive factors for Mortality among non-HIV-infected patients with Pulmonary Tuberculosis and Respiratory Failure

S.M. Lin, T.Y. Wang, W.T. Liu, C.C. Chand, H.C. Lin et al. *Int J Tuberc Lung Dis* 2009; **13(3)**: 335-349

The objective was to determine predictive factors for mortality among pulmonary tuberculosis (PTB) patients without human immunodeficiency virus (HIV) infection and in need of mechanical ventilation (TBMV). From July 2004 to December 2005, 612 respiratory failure patients requiring mechanical ventilation were admitted to the intensive care unit (ICU) of Chang Gung Memorial Hospital, Taipei, Taiwan. Of these, 59 non-HIV-infected patients had active PTB as the primary cause. Mortality rates were measured in TBMV patients and predictors were investigated. Incidence of treatment delay for nosocomial pneumonia was compared between survivors and fatalities. Of the 59 patients with TBMV, 40 (67.8%) died in the ICU. Multi-organ failure syndrome (OR 8.59, 95%CI 1.85-101.27) and nosocomial pneumonia (OR 5.77, 95%CI 1.33-44.36) were independently associated with in-hospital mortality. Treatment delay of more than 24 hours for nosocomial pneumonia was significantly more frequent among fatalities than among survivors (19/26, 73.1% vs. 0/3, 0%; *P* = 0.033). Nosocomial pneumonia in TB patients with respiratory failure is associated with a poor prognosis; this appears to be further aggravated by delays in appropriate treatment. Measures to prevent nosocomial pneumonia should be carefully instituted and treatment for nosocomial pneumonia should be started promptly among such patients.

Radiographic manifestations of culture-positive Pulmonary Tuberculosis: Cavitary or non-cavitary?

J.A. Al-Tawfiq and B.M. Saadeh. *Int J Tuberc Lung Dis* 2009; **13(3)**: 367-370.

The objective was to examine the radiographic pattern of patients with pulmonary tuberculosis (PTB) in Saudi Arabia and the relation of these findings to demographic and microbiological data. It was a retrospective hospital-based series of patients with culture-positive PTB. Among 168 cases of culture-positive PTB identified, 97 (57.7%) were males and 71 females (42.3%); 136 (81%) were Saudis and 19% were non-Saudis. The mean age was 52.3 ± 19.2 years: nine (5.4%) were children aged d”18 years and 64 (38.1%) were adults aged >60 years. Overall, 121 (78%) had upper lobe infiltrates, 35 (19.7%) had cavitary lesions and 33 (19.6%) had both upper lobe infiltrate and cavitation. Lymphadenopathy and pleural effusion were each present in 11.3% of the patients. Patients aged >60 years were less likely to have upper lobe infiltrate (38/64, 59.4%) compared to children (7/9, 77.8%) and adults aged 19-60 years (76/95, 80%, *P* = 0.001). Diabetes mellitus was documented in 57/135 (42.2%) patients. There was no difference in the presence of upper lobe infiltrate and the presence of cavitation in patients with and without diabetes mellitus. Cavitary or upper lobe infiltrate remains a common presentation of PTB. As patients aged >60 years often present with no cavitation and without upper lobe infiltrate, it is important to keep in mind the possibility of tuberculosis in this group of patients.

Analysis of discordance between the Tuberculin Skin Test and the Interferon-Gamma Release Assay

A. Machado Jr., K. Emode, I. Takenami, B.C. Finkmoore, T. Barbosa, J. Carvalho et al. *Int J Tuberc Lung Dis* 2009; **13(4)**: 446-453.

The objective was to analyze factors associated with discordance between tuberculin skin test (TST) and interferon- gamma release assay (IGRA) results among household contacts of pulmonary tuberculosis (PTB) patients. TST (purified protein derivative) and IGRA (QuantiFERON®-TB Gold) were performed on house- hold contacts of PTB patients diagnosed between 2006 and 2007 in Salvador, Brazil. Discordant test groups were compared with the TST-/IGRA- group. Of 261 household contacts satisfactorily tested by TST, 145 (55.6%) had positive TST results; of 298 satisfactorily tested by IGRA, 127 (43.1%) had positive results. The test agreement was 0.76 (• = 0.53, 95%CI 0.43-0.63). Sixty-one (24%) were discordant: 44 (72%) with TST+/IGRA- and 17 (28%) with TST-/ IGRA+ results. Compared to the TST-/IGRA- group, the TST+/IGRA- and TST+/IGRA+ groups were significantly more likely to have a chest X-ray showing old lung scars (OR = 6.8, 95%CI 1.3-35.0; OR = 7.4, 95%CI 2.2-24.4, respectively). The TST -/IGRA + group was exposed to their index cases for significantly longer than the TST-/IGRA- group (OR = 7.2,95%CI 1.7-29.3). The TST+/IGRA- and TST+/IGRA+ groups shared more similar characteristics with each other than with the TST-/IGRA- group. In a setting endemic for TB, TST results appear to be more suitable in the decision to treat latent TB infection.

Risk factors for new pulmonary tuberculosis patients failing treatment under the Revised National Tuberculosis Control Programme, India

R. Singla, D. Srinath, S. Gupta, P. Visalakshi, U.K. Khalid, N. Singla et al. *Int J Tuberc Lung Dis* 2009; **13(4)**: 521-526.

The objective was to study the risk factors for new pulmonary TB (PTB) patients failing treatment. It was a prospective case-control study. The profile of new PTB patients failing treatment (i.e., sputum smear- positive at 5 months of treatment) and responders under the Revised National Tuberculosis Control Programme (RNTCP) were compared and risk factors associated with treatment failure were analysed. A total of 42 treatment failure cases and 76 controls were enrolled in the study. The presence of cavity on chest X-ray (CXR), sputum acid-fast bacilli (AFB) smear positivity at 2 months of treatment and the number of interruptions in treatment were independently associated with failures. Among failure patients at 5 months, 17 (40.5%) had negative sputum culture for *Mycobacterium tuberculosis*, and only six (14.3%) had multi- drug-resistant TB (MDR- TB). When put on re-treatment, patients with smear-positive, culture-negative sputum had cure rates of 88.2% compared to 28.6% among culture-positive patients. The presence of cavity on CXR, sputum smear positivity at 2 months of treatment and the number of interruptions of treatment are risk factors for failure. Among failures based on smear examination, the prevalence of MDR- TB is low and many patients have negative cultures for *M. tuberculosis.* Smear positivity at the end of treatment may not be a reliable indicator of treatment failure.

Effect of Tripod Position on Objective Parameters of Respiratory Function in Stable Chronic Obstructive Pulmonary Disease

S.P. Bhatt, R. Guleria, T.K. Luqman-Arafathl, A.K. Gupta, A. Mohan, S. Nanda1 and I.C. Stoltzfus. *Indian J Chest Dis Allied Sci* 2009; **51(2)**: 83-85.

The objective was to examine changes in respiratory dynamics in patients with chronic obstructive pulmonary disease (COPD) sitting leaning forward with hands supported on the knees (tripod position), a posture frequently assumed by patients in respiratory distress. Spirometry, maximal inspiratory and expiratory pressures (MIP and MEP) generated at the mouth, and diaphragmatic excursion during tidal and vital capacity maneuver breathing measured by B-mode ultrasonography were studied in 13 patients with stable COPD in sitting, supine and tripod positions. Mean±SD age of patients was

52.2±6.8 years. Median disease duration was three years. There was no statistically significant difference in spirometry for sitting, supine and tripod positions (FEY_1: 1.11±0.4L, 1.14±0.5L and 1.11±0.4L p=0.99), respectively, (FEY_1/FVC: 49.2±11.0, 53.7±8.5 and 48.5±11.3, p=0.37), mouth pressures (MIP: 102.9±28.9, 90.6±29.1 and 99.2±32.9 cm H_2O, p=0.61 and MEP: 100.8±29.9, 100.4±34.4 and 90.6±32.6 cm H_2O, p=0.74) and diaphragmatic movements during tidal (16.1±5.9, 20.1±6.8 and 16.6±6.2 mm, p=0.22) and forced breathing (33.9±11.0, 43.1±19.6 and 37.4±17.1 mm, p=0.35). Commonly measured indices of respiratory function were not different in the tripod compared to sitting and supine positions.

Errata

	Name of the author printed as	Names of the authors to read as
April, 2009 issue Page 108	K. Gowrinath	K. Gowrinath and Rahul Magazine

Indian J Tuberc 2009; 56:167-170

GUIDELINES FOR CONTRIBUTORS

GENERAL

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

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

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

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e.g. Jain NK, Chopra KK, Prasad G. Initial and Acquired drug resistance to Isoniazid and Rifampicin and its implications for treatment. *Indian J Tuberc* 2002; **39**: 121-124.

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