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Indian Journal of Tuberculosis

Vol. 59 **New Delhi, October, 2012** **No. 4**

Editorial

IS XDR-TB A SUB-GROUP OF MDR-TB? NEED TO REORGANIZE ALPHABETS AGAIN!

[*Indian J Tuberc 2012; 59:* 187-189]

The successful treatment regimen for tuberculosis was the most important paradigm in tuberculosis (TB) history. From the difficult journey through cure by regal touch, cod liver oil, surgical collapse therapy and sanatorium management, we advanced to successful short course chemotherapy in the hope to eradicate the disease. However, availability of a number of limited drugs and their misuse, instead, resulted in emergence of drug resistance (DR) and tuberculosis control is now becoming a serious challenge; heralding a return to the pre-antibiotic era. There is neither a comprehensive understanding of magnitude of this problem nor about the characteristics of resistance pattern. Terminologies used for drug resistance in tuberculosis either to isoniazid (H) and rifampicin (R) or other second line drugs are actually becoming a soup of alphabets. Resistance to H and R as MDR (multidrug resistance), resistance to H, R with monoresistance to either fluoroquinolones (FQ) or amino glycoside (AM) as pre-XDR (pre-extensive drug resistance), resistance to H, R with FQ and AM as XDR (extensive drug resistance), resistance with all first and second line drugs as XXDR (extremely drug resistance), resistance to all available anti-TB drugs as TDR (total drug resistance)!. Unfortunately, no nomenclature in drug resistant TB is yet the final product and the alphabet soup needs to be reorganized.

MDRTB treatment is standardized under programmatic guidelines for management of multidrug resistant TB (PMDT).[1] An integrated algorithm for drug resistant TB treatment under these guidelines is a standard category (CAT)- 4 regimen comprising kanamycin (KM), ethionamide (Eto),cycloserine (Cs), leofloxacin (Lvx), ethambutol (E), and pyrazinamide (Z). When DST results are available, there is provision to modify therapy in case of monoresistance to Ofx or Km; with substitution of Lvx by para-aminosalicylic acid (PAS) and moxifloxacin (Mfx) and for KM resistance substitution with capreomycin (Cm). In case of resistance to both Km and Ofx, it is labelled as XDR-TB and treatment under CAT-5 given as a salvage regimen, comprising Cm, PAS, Mfx, lenezolid (Lzd), high dose INH (High dose H), amoxicillin-clavalunic acid (Amx/Clv), clofazimine (Cfz).

Studies have shown high resistance to quinolones as responsible factor for poor culture conversion in MDRTB patients.[2] Also with regular practice of giving aminoglycosides (AM) and fluroquinolones (FQs) for treating bacterial infections makes us presume high likelihood of resistance to these drugs. Pilot survey of antibiotics from India showed the highest sale of quinolones followed by amino glycosides.[3] Surveillance studies showed resistance to newer antibiotics such as FQs in all areas, with the rates being high in India.[4] Ofx resistant isolates are consistently shown to be cross-resistant to newer FQs and FQ DST along with first line DST in areas with high rates of MDRTB has been recommended.[5] Treatment outcomes and survival based on DST pattern in MDRTB showed that mono resistance either to FQ or AM, was independently associated with poor outcomes in patients with MDRTB and also suggested implementation of strategies to identify and cure these patients.[6] For this reason, early DST for FQ and AM is mandatory before designing a regimen for MDR TB patients.

DST results for various second line drugs are not reliable as existing tests are not standardized and are less reproducible than results for the first line drugs and have poor clinical predictive values[7]. This has been thoroughly studied and consensus reached on appropriate methods, critical drug concentrations that define resistance, reproducibility of testing and reliable DST results are available for H, R, Ofx, and KM but the same not true for other anti-TB drugs.[8] It was proposed that in settings where XDR is a concern, DST for HR and AM, FQs should be merged in order to enable the rapid identification of XDR-TB patients.[9] Ignorance of timely detection of resistance may compromise the effectiveness of global disease control. Further, with early availability of DST results, simplification and standardization of regimens would make treatment more practicable.

Mumbai has adopted criteria C for referral for DST[1] (all patients should be referred for DST at diagnosis of TB except new smear positive and smear negative patients at diagnosis without HIV infection) in view of increased burden of MDR TB cases. In our experience at PMDT site, there are many patients already treated with multiple second line drugs. Accredited laboratory capacity in our country is limited. For Mumbai, National Tuberculosis Institute (NTI) being the only accredited laboratory to do second line DST (Ofx, Km), there is long waiting period for the DST results. As per recent PMDT updated guidelines, baseline DST for second line drugs is mandatory. However, Mumbai does not have this provision yet. Scale up of global laboratory capacity for culture DST was considered under global plan to STOP TB also.[10]

Standard treatment under guidelines is appropriate in second line treatment naive patients only. However, when treated with second line drugs earlier, every individual patient presents with their own set of challenges. While designing a regimen, it is essential to be meticulous in obtaining the history of past treatment. A systematic review concluded previous treatment with second line drugs being the strongest risk factor for resistance to various second line drugs and a responsible factor for fourfold increased risk of XDR TB and suggest stratification of used drugs.[11,12] World Health Organization (WHO) also recommends availing drug resistance data and patient treatment history when designing a regimen. With the clarity of detailed history of drugs taken in the past and DST results, we will be able to realize that so called XDR-TB cases will actually turn out to be a subgroup of MDR-TB having dual resistance (Ofx and Km) which also can be treated with modified CAT- 4 regimen with replacement of Km with Cm and Lvx by PAS in addition to other standard CAT- 4 drugs. Cases that have failed on second line therapy Cat 4 or modified Cat 4 can be labelled as XXDR-TB which are difficult to prove due to non-reliability of second line drug DST other than AM and FQ. These may be treated with salvage drugs. XXDR-TB cases that have failed on salvage drugs are likely to be TDR cases. There is a concern regarding exponential rise in evolution of XDR cases into XXDR-TB cases, if these patients are not handled cautiously.

For patients not treatable by standard or modified standard therapy and salvage regimen, in any case, question always remains… what next? In order to eradicate tuberculosis, it is vital that we understand our mistakes today itself. Knowing the fact that single drug (addition syndrome) should not be added to failing regimen, we should not force ourselves to treat these patients irrationally just to not to deviate from programmatic guidelines. A silent malnutrition crisis in the city adds to the imbalance in people's access to resources and intolerance to drugs that affects treatment adherence. Added to this are problems like immigrant population and limited manpower creating constraints for monitoring and follow up of these patients during therapy. There is a strong need to isolate patients of XXDR–TB with rebuilding of sanatoria along with infection control measures, reduced hospitalization time and outpatient treatment facilities. An epidemiological modeling study from south Africa[13] suggested the combination of mask use with reduced hospitalization time and a shift to outpatient therapy could prevent nearly a third of XDR tuberculosis cases. New classes of anti-TB drugs such as TMC 207 and delamanid may be available in the near future.

Even if new drugs are available, they may be rapidly "burnt" as a result of clinical and public health malpractices similar to some of the key old drugs.[14] A strict national policy for their rational use therefore should be in place. Failure to act now to contain the threat posed by these patients will have devastating consequences.

Vinaya S. Karkhanis and Jyotsna M. Joshi*

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* Department of Pulmonary Medicine, T.N. Medical College and B. Y. L. Nair Hospital, Mumbai (Maharashtra)
Correspondence: Dr. J. M. Joshi, Professor and Head, Department of Pulmonary Medicine, T.N. Medical College and B. Y. L. Nair Hospital, Mumbai – 400008 (Maharashtra); Phone: 022-23081490; E-mail:drjoshijm@gmail.com

Editorial

TOTALLY DRUG RESISTANT TUBERCULOSIS - A FACT OR MYTH?

[*Indian J Tuberc 2012; 59:* 190-193]

Recently there was a hue and cry over the publication of an article about the existence of totally drug resistant tuberculosis (TDR-TB).[1] It was more of a media outcry rather than being of any major scientific significance. The Government of India also over-reacted, possibly rightly so, because of obvious public health importance and public pressure. Subsequently, a number of other editorials and correspondences appeared in different scientific journals.[2-4] The question to be answered is whether the scientific existence of TDR-TB is a reality or a myth? It is possible theoretically that there may be some cases of resistance to some or all of the second line drugs in India and many other high burden countries because of widespread misuse of second-line drugs in the private sector and prescription by physicians who are not supposed to do so. The authors have highlighted the possible causes of such a development of TDR in four of their patients. Detection of drug resistant cases, particularly in public health settings, requires the availability of good quality-assured and accredited laboratories. Unfortunately, in India, there is a lack of such accredited laboratories in sufficient numbers to get an accurate idea of the extent of drug resistance in the country. This is true for many other developing, high-burden countries also. In India, the Revised National TB Control Programme has planned to have 43 such accredited Intermediate Reference Laboratories (IRLs) throughout the country both in the public as well as private sectors to undertake the culture and DST for early diagnosis of MDR cases using solid LJ media with some laboratories having facilities for liquid culture for mycobacteria with DST for first line drugs only. These laboratories have only accreditation for rifampicin and isoniazid DST and in a few, with facility for additional ethambutol and streptomycin. So far, the country has 31 such laboratories and others are at various stages of preparation. The laboratory that reported the so-called TDR cases is a private sector hospital accredited for first-line anti-tubercular drug susceptibility testing only by the National Programme. Although the laboratory might be doing second line drug testing like many other laboratories in the country are doing, it is not accredited for DST for these second line drugs.

The laboratories in India that are accredited for second-line drug testing are two National Reference Laboratories: the National Institute for Research in Tuberculosis, Chennai and the LRS Institute of Tuberculosis and Respiratory Diseases, New Delhi and recently, the National Tuberculosis Institute, Bengaluru. The country has also other accredited laboratories for molecular testing using LPA, and the Gene Xpert is undergoing field-testing for its utility in the country. The Central TB Division of the Government of India is rapidly expanding the DOTS-Plus programme in the country to cover a maximum number of cases with MDR tuberculosis with special emphasis on quality DOTS programme to prevent the development of MDR TB. Almost all states of India are having Programmatic Management of Drug Resistant Tuberculosis (PMDT) services in some districts with 414 million (35%) population in 211/659 (32%) districts having access to these services. It is important that accredited and quality-assured laboratories are the need of the hour. WHO states that the term “totally drug resistant” has not been clearly defined for tuberculosis because there is no reliable method to diagnose it through drug susceptibility testing (DST). And importantly, correlation of DST results with clinical response to treatment has not yet been adequately established. The prognostic relevance of *in vitro* resistance to drugs without an internationally accepted and standardized

drug susceptibility test therefore remains unclear, and current WHO recommendations advise against the use of these results to guide treatment. Therefore, report of four TDR cases by the said hospital needs reconfirmation by accredited laboratories, particularly when the WHO has reservations about the use of such a term. It is a well-known fact that DST of most of the second-line drugs is very difficult and the reliability is always questionable, except when done by competent and efficient laboratories. In fact, in January, 2011, one of the National Referral Laboratories of the country had reported that about 1.34% of their total MDR cases seen over a period of three years were resistant to all the second-line drugs i.e. panresistant.[5] The authors have carefully avoided using the term TDR, rather they have mentioned these cases to be panresistant to the available tests. Moreover, the data was from a tertiary care hospital setting not reflective of what is happening at the community level. Even a year earlier than this report from New Delhi, the Supra National Reference Laboratory, Chennai had reported the culture and drug susceptibility testing results of 2816 tuberculosis patients from across India who had failed repeated treatments from 2001 to 2004.[6] Of 1498 (53%) identified as having MDR-TB, 671 (44.8%) were resistant to ≥1 second-line drugs with 490 (32.7%) to ethionamide, 245(16.4%) to ofloxacin and 169(11.3%) to kanamycin; 69(4.6%) were XDR-TB. This was again from a highly select and non-representative patient group. Another report earlier studied the *Mycobacterium tuberculosis* isolates from a representative sample of new and previously treated smear-positive pulmonary TB (PTB) cases from Gujarat who were subjected to drug susceptibility testing (DST) against first-line drugs at a World Health Organization supranational reference laboratory (Chennai). Isolates found to have at least both isoniazid (INH) and rifampicin (RMP) resistance (i.e., multidrug-resistant TB [MDR-TB]) were subjected to second-line DST. Of 1571 isolates from new patients, 1236 (78.7%) were susceptible to all first-line drugs, 173 (11%) had any INH resistance and MDR-TB was found in 37 (2.4%, 95% CI 1.6-3.1). Of 1047 isolates from previously treated patients, 564 (54%) were susceptible to all first-line drugs, 387 (37%) had any INH resistance and MDR-TB was found in 182 (17.4%, 95% CI 15.0-19.7%). Among 216 MDR-TB isolates, 52 (24%) were ofloxacin (OFX) resistant; seven cases of extensively drug-resistant TB (XDR-TB) were found, all of whom were previously treated cases.[7]

Thus data is available from this country from time to time, but the report was sensationalized by lay media, which is not desirable from a public health point of view. A similar report on TDR-TB was reported from Iran in 2009 also[8], but the world had given little attention to that. In fact after the report from Mumbai, WHO held a special meeting in Geneva in March 2012 and, after due deliberations, concluded that the term "TDR-TB" could not yet be recommended as an entity because of the fact that drug susceptibility testing for drugs other than those that define XDR was problematic to interpret. DST results for different second line drugs are not reliable and difficult to interpret as existing tests are not standardized and are less reproducible than results for the first line drugs and have poor clinical predictive values.[9] This has been thoroughly evaluated and consensus has been reached on appropriate methods, critical drug concentrations that define resistance, reproducibility of testing and reliable DST results are available for isoniazid, rifampicin, ofloxacin, and kanamycin but the same is not true for other anti-TB drugs.[10-13] It was proposed that in settings where XDR-TB is a concern, DST for these drugs should be merged in order to enable the rapid identification of XDR-TB patients.[14] Interpretation of DST for Pyrazinamide is further, a complicated issue.

It is well-recognized that quality assurance and accreditation are now key issues in determining the culture and DST for mycobacteria. Although a number of unregulated and unqualified laboratories in the country carryout these tests, they are not reliable nor acceptable as they have not undergone accreditation process. The same has been emphasized time and again by WHO as well as the RNTCP of India and the programme is making all efforts and investing hugely both monetarily and technologically to develop such laboratories in the country.

The laboratory that first reported these cases is only accredited and qualified for rifampicin and INH DST; then it is not clear how these results will be taken as their face value and one will have some reservation in accepting their DST results for second line anti-TB drugs. Further, the laboratory has undertaken Hain Genotyping MTBDR Plus and MTBDRSl. The latter is not yet even approved and accepted by WHO for gyrA and rrs mutations, leave alone their validity in Indian setting.

The RNTCP, India has the provision of treating drug resistant cases (particularly XDR-TB cases) using Category V drugs that include capreomycin, moxifloxacin, linezolid, amoxicillin-clavulanic acid, clofazimine, PAS, high dose isoniazid, and clarithromycin. Although the experience is not very large in this country about the efficacy of these drugs, Singla *et al*[15] have reported the results from a small number of 29 cases of MDR-TB treatment failure patients using some of these drugs. 89.7% of these patients achieved sputum smear and culture conversion; 72.4% showed interim favourable outcome; 10.3% died, 6.8% failed and 10.3% patients defaulted. Similar experience has also been reported by others.[16]

Nonetheless, the report has highlighted the issue of drug resistance in India that includes the possibility of resistance to many drugs and the problem of wrong treatment by some private practitioners, private and corporate hospitals. The authors have rightly pointed out that patients with MDR tuberculosis only should be treated within the confines of government sanctioned DOTS-plus programmes to prevent the emergence and spread of this form of tuberculosis. However, the authors should not have used the term "TDR-TB", particularly when their laboratory is not even accredited to carry out such DST for second-line anti-TB drugs. Further, genotypic DST analysis for various drugs carried out by the laboratory has not even been validated.

D. Behera*

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* Professor, Department of Pulmonary Medicine, PGIMER, Chandigarh and Ex-Director, LRS Institute of Tuberculosis and Respiratory Diseases, New Delhi; Email:dirlrsi@gmail.com, dbehera@glide.net.in

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Review Article

DRUG DISCOVERY IN TUBERCULOSIS: A MOLECULAR APPROACH

Partha P. Mitra*

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Summary: Despite unquestionable success of the combination drug therapy, tuberculosis (TB) very recently has drawn major attention because of the global upsurge of MDR-TB, XDR –TB and HIV-TB co-infection cases. In the last four decades, only one compound is added to the treatment regimen leaving ample opportunities to find out a new generation of TB drugs. The modern concept of drug discovery utilizes the integrated knowledge of genomics, proteomics, molecular biology and systems biology to identify more specific targets. The purpose of this review is to revisit the field of tuberculosis drug discovery based on those new concepts to identify novel targets. **[*Indian J Tuberc 2012; 59:* 194-206]**

INTRODUCTION

TB had been considered as one of the most fatal diseases to the human race long before Robert Koch discovered the staining technique to detect the responsible bacilli *Mycobacterium tuberculosis (M.Tb).* From the beginning of seventeenth century until World War-II, several parts of Europe, America and Japan suffered from the TB epidemic with the consequence of death of several million people[1]**.** However, a major breakthrough in the TB treatment came after introducing streptomycin, followed by *p*-aminosalicylic acid (1949), isoniazid (1952), pyrazinamide (1954), cycloserine (1955), ethambutol (1962) and rifampin (rifampicin; 1963) as an effective primary line of anti-TB drugs. Single drug treatment in TB has however, several drawbacks which include prolonged treatment time to completely eradicate the bacteria as well as increasing the opportunity to develop drug- resistant TB bacilli which has been documented in almost every country where the disease is prevalent. Although pulmonary TB related death has declined sharply in the western world since the beginning of the 20th century, it still causes major health hazards, especially in Asia, Africa and the Western Pacific region. In the beginning of 1980s, increase in TB related mortality raised a global health concern mainly because a) drug-resistant Mycobacterium and b) opportunistic infection of TB bacilli in the immunodeficiency condition as a result of HIV infection. Present gold-standard treatment for TB is a six-month course of rifampicin and isoniazid, supplemented in the initial two months with pyrazinamide in association with either ethambutol or streptomycin. Persons with latent TB infection are treated with isoniazid for six months [2]**.** The current success rate using cocktail drug is about 95% and is effective on drug-susceptible *M.Tb* provided the patient completes the six to nine month treatment period. Fixed dose combination (FDC) is one of the WHO recommended optimal drug treatments for TB in order to reduce treatment time, cost and perhaps to reduce the risk of emergence of drug resistant mycobacterium [3]. However, any difficulties of FDC treatment can generate chronically contagious cases, which may excrete drug-resistant mycobacteria [4]. The Directly Observed Treatment Short-course (DOTS) and DOTS-Plus programmes are recommended by WHO to control TB by providing a comprehensive organizational and infrastructural framework for the rational use of diagnosis, drug supply, as well as case and programme management services. DOTS-Plus accommodates additional second-line TB drugs to the people presumed to get MDR-TB [5]**.** Recently, TB due to Multi drug resistant (MDR-TB) and extensively drug resistant (XDR-TB) strains gave a wakeup call to the global health organization agencies to organize the TB programme and reinstate a fresh effort to develop a new drug against the pathogen. According to WHO, about 650,000 cases of MDR-

*Senior Research Officer, Research Wing, Diamantina Institute, Princess Alexandra Hospital, University of Queensland, Level 4, 20 Cornwall Street, Woolloongabba, Queensland - 4102 (Australia).

TB have been reported worldwide in 2010, among them 9% are estimated to be XDR-TB cases. At present, globally, the fatality rate due to MDR-TB infection is about 30%. A syndemic relationship has been reported in HIV and MDR-TB. In 2010, about 3,50,000 people died of HIV-associated TB and 25% of deaths due to HIV are associated with TB[6]. The global scenario of TB related health hazard is not only pointing to a major loophole of the management of TB programme but also indicating an urgent need to develop new drugs or new combinations of drugs to save the lives of millions of people worldwide. This review aims to shed some light on to redirect our approach to discover a new TB drug by utilizing recent advancements in this field.

CURRENT OPINION ON TB DRUG DEVELOPMENT

TB treatment has been standing in the same strategic point which was started almost five decades ago. Cocktail drug therapy, the most widely accepted TB treatment, though effective, but has a prolonged treatment time and is inefficient to control disease progression in MDR-TB and HIV-TB co-infected patients. Current trends in anti-TB drug development are mainly driven by three major factors: a) development of drugs with long lasting antimycobacterial activity *in vivo* is desirable as because it will be helpful to bypass the problem of non–adherence and thus can reduce the risk of emergence of MDR and XDR-TB cases, b) development of novel compounds to combat MDR-TB and XDR-TB is urgently needed. c) a new class of anti-TB drugs will be very promising for prevention of latent infection or eradication of slowly metabolizing or dormant populations of mycobacterium bacilli [7,8]**.** Companies investigating on new anti-TB drugs have been deterred by a number of constraints which include a) insufficient animal models that closely mimic the human TB; b) difficulties in demonstrating the obvious benefit of a new anti-TB agent over pre-existing drugs, since multi-drug combination therapy is highly effective against ordinary cases of TB; c) perceived lack of commercial return to companies as the disease is mostly prevalent in developing countries and the cost is less than US$13 for the whole course of treatment. Anticipating the success of combination therapy, more reports are coming out in the last few years addressing the efficacy of such therapeutic approach. For example, clinical studies carried out very recently in South Africa with patients suffering from drug sensitive and MDR-TB showed a promising result. A combination of PA-824{(6S)-2-nitro-6-{[4-(trifluoromethoxy)benzyl]oxy}-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine}-moxifloxacin-pyrazinamide has been shown to be very effective in reducing the early bacterial count in the sputum of the treated patient[9].Global TB Alliance and Bayer AG very recently replaced Isoniazid by Moxifloxacin in the cocktail of the combination drug therapy which is reported to reduce the treatment time by two months in mice[10]. Currently, this combination is in human clinical trials in several geographical regions around the world including the cases of HIV-TB deadly co-infection and results are expected soon. At present, a combination of antiretroviral therapy and rifampin has been considered as the back bone of treatment of HIV-TB cases[11]. Such efforts to improve the efficacy of combination treatment regimen created a novel example of low-risk drug discovery profile rather than focusing on to develop a completely new stand alone molecule against TB which should be either more cost effective than the existing drug and/or reduce significantly the treatment time. In spite of the success of combination therapy in the field of TB, several new drugs are currently in the development pipeline to come out as a single drug therapy in TB treatment. Among them, novel drugs such as TMC207, SQ109 and sudoterb [LL3858] have produced most promising results. Besides that, gatifloxacin and moxifloxacin; linezolid, PNU100480 and AZD5847; metronidazole, OPC-67683 and PA-824 are currently considered as very promising next generation TB drugs[12,13]. According to the latest WHO global report, list of such drugs and their status in the clinical trials are summarized in Table-1. Though the initial reports of these current pipeline of drugs are very promising, but the increasing global threat of MDR-, XDR-TB and HIV-TB have created a new niche for the development novel drugs in this field. Therefore, updated knowledge in the field on novel compound sources, methods of target validations, molecular models and animal proof of concept, which will be discussed in next several sections, can be implemented to find new drugs in the field of TB.

Table 1: List of latest drugs* and their status in the clinical trials.

Phase-I	Phase-II	Phase-III
AZD5847	TMC-207 OPC-67683 PA-824 Linezolid Rifapentine SQ-109 PNU-100480 Novel Regimens[a]	Gatifloxacin Moxifloxacin Rifapentine

* They are used as single drug or in combination or used as a replacement drug in the existing cocktail therapy.

NOVEL COMPOUND RESOURCE

Classically, any drug discovery process starts with the finding of a set of novel compounds, considered as hits, from a compound collection defined as library. Such a kind of library is built up based on collection of compounds from a wide variety of sources which may include small molecules with novel scaffold and unknown function, molecules originated from natural sources like secondary metabolites of plants and marine habitats. Recently, researchers are utilizing libraries with several thousands molecules of defined structure for the identification of hits in the field of targeted drug discovery. For example, the crystal structure of any key enzyme can be utilized for precise designing or understanding of the scaffold of competitive inhibitors. Similarly, interaction between two proteins either essential for host pathogen interaction or to drive any key metabolic pathway can be targeted to design a molecule which can interfere with the interaction. In silico searching, either competitive inhibitors for any enzyme or inhibitors of protein-protein interaction in any such library is now-a-days a routine job for drug discovery research. Until recently, not too much attention was paid to the natural sources, for example, marine sources, to search for novel compounds against Mycobacteria. There are several unique reasons why marine resources should be explored for searching novel compounds such as a) very wide biodiversity and largely unexplored environment (80% of all life forms on Earth are present only in the oceans), b) uniqueness and diversity at the genetic level (Sponges: >100 000 genes and Humans: ~25 000 genes). Two commercially available antivirals Ara-A (acyclovir) and AZT (zidovudine) were isolated from sponge[14] and c) large chemical diversity (marine organisms often incorporate halogens like F, Cl, Br, I into their chemical structures which are rarely seen in terrestrials). Marine organisms are also a rich source of a very wide variety of bio-reactive molecules like phenols, terpenoids, fatty acids, polysaccharides, proteins, acetylenes, terpenes, indole derivatives, and antimicrobial peptides. Several such compounds like saturated 2-methoxylated FA from sponges and secondary marine microbial metabolites like pseudopyronines were evaluated successfully for growth inhibitory effect of mycobacterium [15, 16]. In collaboration with academic institutions, several companies are searching for novel antimycobacterial agents from a repository built up with the extract of wide variety marine organisms. Besides searching for novel compounds against mycobacterium from natural products and secondary metabolites, genetic chemistry may be a completely new generation source to synthesize novel compounds with unique scaffolds. Several natural products like alkaloids, terpens, alkanes, alkenes, alkynes, phenolics and acetogenic quinones have shown antimycobacterial activity [17]. As a stand alone compound, they showed mild activity but became a potential agent when they were put in combination [18, 19]. Taking the advantage of Genetic Chemistry technology, we can synthesize several of such small molecules which are not exposed to the nature. Genes involved in the pathway of producing small molecules can be collected from a wide variety of different species like sponges, bacteria, human, fungi and plants and will be allowed to go through recombination *in vivo*. By combining and evolving genes from different organisms, genetic chemistry has laid a novel *in vivo* platform of combinatorial chemistry and enables the discovery of unique and novel chemical identity which never existed in nature. Drug discovery companies are currently working on several disease models as a target to utilize this technology[20].

TARGETS IN ANTI-TB DRUG DEVELOPMENT

Very recently, several attempts were made to identify new molecules against TB utilizing a wide variety of model systems which include inhibitors of enzymes critical for bacterial metabolic pathways, cell wall synthesis inhibitors, signaling pathway inhibitors[21-25]**.** However, several such attempts are no doubt far less than sufficient and required more attention to develop a new therapeutic approach utilizing the genomic as well as the systems biology information, as drug resistant TB and TB in a HIV patient created a new paradigm of global health concern. A successful drug discovery programme against TB should include a novel molecular model system which will be unique to the bacteria and should be targeted independently without affecting the host system. Targeting enzymes in a metabolic pathway is a classical approach to inhibit the bacterial growth but the major limitation here is frequent development of resistance. Isoniazid, an inhibitor of the mycolic acid pathway enzyme InhA (an enoyl reductase), is used for the primary line of TB therapy. Patients who undergo treatment for the MDR-TB have shown a very high rate of mutation in this enzyme [26, 27]**.** Other enzymes such as FabH (â-ketoacyl-acyl carrier protein synthase III) that play a very important role in mycolic acid biosynthetic pathway, have drawn recent attention as a fresh drug target[28]. Using structure-based drug designing technology, it is possible to develop very high affinity inhibitors which can be helpful for circumventing the normal mechanism of mutation mediated drug resistance [29]**.** Cyclopropane synthases have been shown to be implicated in pathogenicity and therefore constitute attractive targets for the development of new drugs against TB [30]. In *M.Tb*, genes cmaA2, mmaA2 or pcaA encode enzymes that are involved in the cyclopropanated mycolic acid synthesis, are well characterized and has not been explored in targeted drug discovery[31]. Latent infection is another major health concern in TB prevalent zones and about one third of the world population is estimated to be latently infected. During this asymptomatic phase of infection, *Mycobacterium* is partly capable of by passing the host immune system with the formation of granulomas[32]. Recent data show two enzymes in the glyoxalate shunt pathway, isocitrate lyase and malate synthase play an important role in the growth, persistence and support granuloma formation in host[33, 34]. Subsequently, those enzymes are considered as targets to carry out highthroughput searches to find out potential new enzyme inhibitors. Although many potential new drug targets have been identified and their number is increasing with time therefore, more effort is required in target validation to show unequivocally that they are specifically acting upon the bacterial growth and survival.

APPROACH TO TUBERCULOSIS DRUG DISCOVERY

During the period of the last two decades, the cost of drug discovery has increased several fold partly to meet the increasingly complicated criteria of the regulatory authority. A recent survey on the costs of the research and development of 68 randomly selected new drugs from ten pharmaceutical firms shows an increase in capital costs at an annual rate of 7.4% above general price inflation[35]**.** Even simple arithmetic suggests that the costs of discovering new chemical entities have been climbed up more than four fold in the last two decades. An attempt at TB drug discovery combines several steps and an exit point should be attached to each step to rationalize the cost of the whole discovery process. The three stage approach for drug discovery and development against *M.Tb* should consist of a) highthoroughput, low cost, time saving assay using nonpathogenic mycobacterium, b) lead optimization using pathogenic organism like *M.Tb* and finally, c) test on appropriate animal model to check *in vivo* efficacy and pharmacokinetics before we reach the stage of human clinical trials. Initial screening, in case of targeted drug discovery, should include a collection of several molecules using optimized reporter based assay system and generated hits will follow lead optimization stage and onwards.

Highthoroughput, low cost time saving assay using non-pathogenic mycobacterium

Mycobacterium can be classified into two major categories depending upon their growth *in vitro*, one is fast growing, non-pathogenic organism and another is slow growing, pathogenic organism.

Slow growing pathogenic mycobaterium will be a difficult organism to screen a large number of targets in a very short period of time and particularly at the same time when one of our main objectives is to develop molecules which will preferentially have antimycobacterial activity. Therefore, a preference was given to use *M. smegmatis* mainly because a) it is non-pathogenic and easy to handle b) growth rate of *M. smegmatis* is almost eight times faster than *M.Tb*, and it is widely used to understand the biology of *M.Tb* such as cell culture, gene expression and persistence in the face of nutrient starvation c) most importantly, *M. smegmatis* was found to display a profile similar to MDR *M.Tb*[36-39]**.** Therefore, cell viability assay could serve as a 'surrogate' for MDR *M. TB*. The main goal of this primary assay is to help in prioritizing the compounds which can be tested further in more specific *in vitro* assays on pathogenic *M. TB*, MDR and XDR strains. There are several reports on the use of *M. smegmatis* in primary screening to select compounds which could be active against *M. TB*[40-43]. It was reported that the susceptibility of *M. smegmatis* for the two frontline anti-TB drugs, isoniazid and rifampicin, was identical to that of MDR clinical isolates of *M. tb*. The specificity of the *M. smegmatis* based on screening has to be extremely specific so that hits generated in this assay can be a potential target for both normal as well as MDR strains.

Targeted drug discovery focusing on validation of the molecular models

Classically targeted drug discovery can be subdivided into several major categories which include a) protein-protein interaction, b) inhibitors of key regulatory enzymes, c) antimicrobial peptides, d) antisense RNA, e) siRNA. Our discussion will be focused mostly on first three categories as others have not yet come out as major potential areas, particularly in the field of TB.

a) *Protein-protein interaction*

A network of protein-protein interaction controls a wide variety of biochemical and cellular processes from bacteria to metazoan. Small molecules impair such interactions have emerged as a drug-like inhibitors and recently several such interactions for a variety of disease models going through the screening process[44]**.** A number of protein-protein interactions (PPIs) network have been proposed for *M.Tb*[45]. Disrupting such PPIs using small molecules will create new opportunities for utilizing non-traditional strategies as therapeutics. A fluorescence based highthroughput protein-protein interaction model has been described in Fig. 1. In this assay, the two interacting proteins were taged with two different fluorescence molecules such as Cyan Fluorescent Protein (CFP) and Yellow Fluorescent Protein (YFP). During interaction, they are close enough to pass the energy from one fluorescent protein to another. In the presence of inhibitors, the energy transfer will be inhibited. This Fluorescence Resonance Energy Transfer (FRET) assay has been considered a routine practice in recent days of drug discovery research.

b) *Inhibitors of enzymes*

As described earlier, enzymes of mycobactrial origin can always be considered as major targets for drug discovery. The major drawback of enzyme inhibitors is that the bacterial cell can easily develop resistance against those inhibitors. At present, several enzymes have been considered as potential targets and some of them are listed in Table-2. Though inhibitors of several enzymes are already tested to check the binding efficiency, however much of the cases can be designed based on the crystal structure of the protein. Active site structure of the enzyme can be determined if the enzyme is crystallized in the presence of a substrate. As well as aiding rational design methods, the detailed structure of the enzyme and the binding position and orientation of ligands allow computer-based drug design methods, such as docking, to be used to screen and identify likely enzyme inhibitors. The basic procedure of computer assisted drug discovery (CADD) has been described in Fig. 2, where virtual hits can be identified by utilizing in silico docking system.

To accomplish docking studies, the native 3D structure of the target protein must be known, typically using X-ray crystallography and virtual

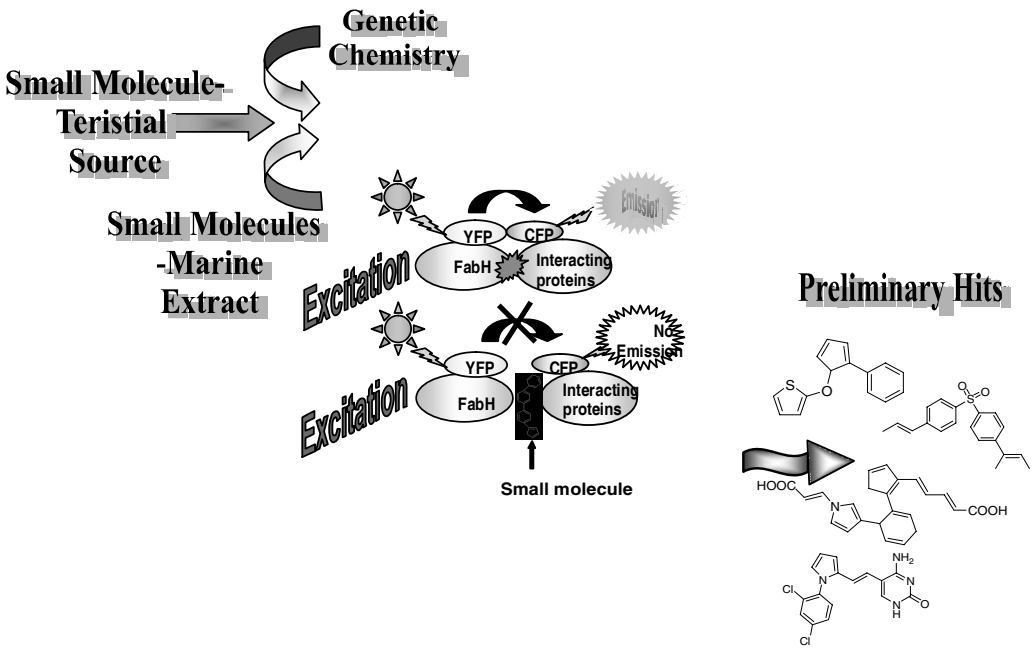

Figure 1: Reverse yeast two hybrid based HT assay models*

*include the mycolic acid biosynthetic pathway enzymes
FRET signal can be generated when two interacting proteins, playing a central role in the mycolic acid biosynthetic pathway, are cloned and overexpressed in the yeast with two different fluorescent tags like YFP and CFP. Such genetically engineered yeast population can be considered as screening machinery to identify novel small molecules originated either from genetic chemistry or from natural sources.

Table 2: List of recent drug targets on mycobacterium

Target	Reference
Maltosyltransferase	Kalscheuer R, *et al*. Nat Chem Biol. May;6(5):376-84. 2010
PhosphoriboPhosphoribosylpyrophosphate Synthetase	Lucarelli AP, *et al*. PLoS One. Nov 15;5(11):e15494, 2010.
ATP phosphoribosyltransferase	ChoY, *et al*, J.lMed. Chem., 51,(19), 2008.
Thymidylate Kinase	Familiar O, *et al*, Chem Med Chem 3,(7),2008
Protein Tyrosine Phosphatase B	Möller-Noren A, J. *et al*, Angewandte Chem. Int. Ed. 47,(32), 2008.
DNA gyrase	Manjunath a, Nuc. Acids Res. 33 (10) 2005.
Cyclopropane synthase	Glickman, MS; Mol. Cell, Vol. 5, April, 2000.
Glycosyltransferases	Lucas, R, Chem Bio Chem, 9, 2008.
Lysine ε-aminotransferase	Dube, D., Med. Chem. Res. 17(2-7) 2008

All recently identified targets and references listed above are enzymes of a wide variety of metabolic activity.

compound libraries can then be docked in the active site. Careful examination of the results will evaluate their binding capacities and allow the discovery of potential inhibitors. An alternate method can be to design inhibitors based on the blue print of the transition state structure. The strongest interaction between the enzyme and the substrate occurs at the transition state of a enzyme substrate reaction and therefore, though it is challenging to identify the intermediate transition state analogues, but it can be an alternative to design a strong binding affinity inhibitor(s) [46,47].

c) *Antimicrobial peptides*

Alveolar macrophages and lung epithelial cells are the first cells that encounter *M. Tb*. Respiratory secretions have microbicidal and microbiostatic properties mediated by their constituent antimicrobial peptides. á- and b-defensins, mainly produced by neuotrophil and various epithelial cells, have been well studied in linking the acquired and innate immunity. The expression of short peptide like defensin is tightly regulated by the NF-*ê*B and requires activation of Toll-like receptor 2 (TLR2)-mediated intracellular signaling pathway. Direct killing of bacilli by antimicrobial peptides as well as to the increased penetration of drugs into the mycobacterium present inside the macrophages, make antimicrobial peptides as potential candidates for the treatment of TB. The effect of combination of antimicrobial peptides and conventional anti-mycobacterial drugs (e.g. isoniazid and rifampicin) has been evaluated against *M. Tb* H37Rv *in vitro*, *ex vivo*, and *in vivo*, and it has been observed that the effective therapeutic dosage of conventional antiTB drugs could be reduced approximately to half by supplementing antimicrobial

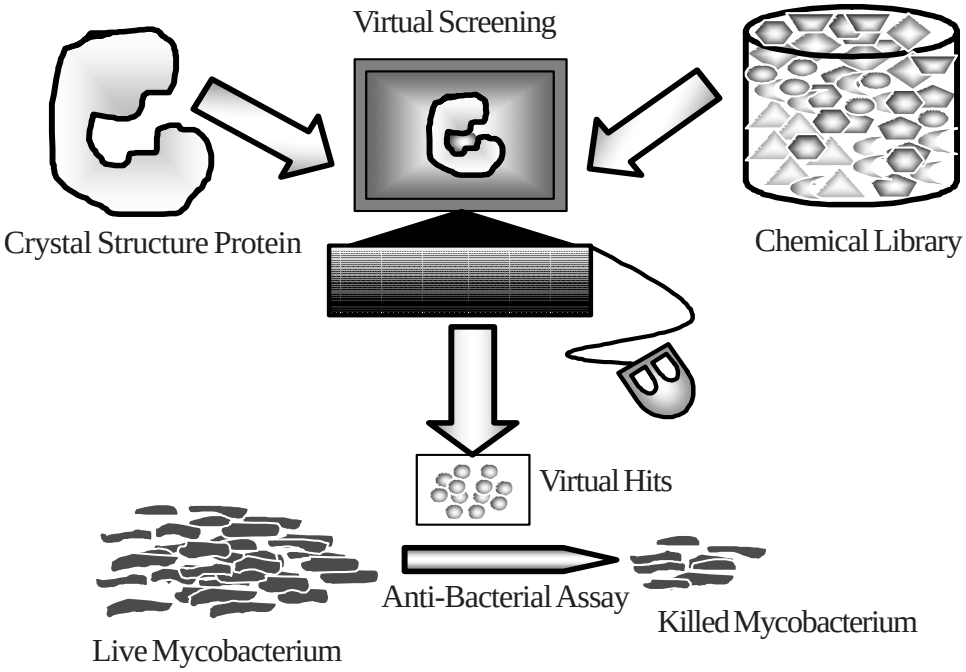

Figure 2: Virtual screening on chemical library using crystal structure of metabolically active proteins

Crystal structure of several crucial proteins of Mycobacterium origin can be used for virtual screening of chemical libraries with indication of antibacterial activity. Such a process, known as molecular docking, may be a novel approach to find out new potential molecules. Screened molecules catalogued based on the binding energy can be tested for antimycrobial activity.

peptides in the therapeutic schedule. Therefore, antimicrobial peptides can be used as adjunct chemotherapy with conventional drugs against human TB[48,49].

LEAD IDENTIFICATION

Monocyte infection Assay

Identification of hits using non-pathogenic mycobacterium should be tested on their pathogenic counterparts to check the efficacy of those compounds. The test is important for lead optimization and validation because several fundamental differences exist between pathogenic and non-pathogenic strains, particularly in metabolic pathways, membrane transportations, and regulation of gene expression as well as more prominently on macromolecules responsible for the pathogenicity. More importantly, all those factors may determine either independently or collectively the response of these organism against a particular molecule with therapeutic potential. For example, *M. Tb* has only five recognizable carbohydrate transporters in the inner membrane, while *M. smegmatis* has twentyeight such transporters at its disposal [50]**.** Similarly, it was found that protein tyrosine phosphatase ptpAt (a virulence factor for *M. tbs* and contributes to its survival within host macrophages) promoter is a highly active in slow-growing species of mycobacteria, such as *M. tb* and *M. bovis BCG*, but inert in fast-growing mycobacterial species, such as *M. smegmatis* [51]. Strong evidence is in favour of the fact that the nature of mycolic acid plays a crucial role in determining the fluidity and permeability of mycobacterial cell wall. *M.tb* modify their mycolic acids by cyclopropanation, whereas fast-growing saprophytic species *M.smegmatis* do not, indicating that this modification may be associated with the adaptation of this organism to oxidative stress[52]. *M. Tb* synthesizes three major types of cyclopropanated mycolic acids through the action of five putative homologous cyclopropane synthases and some of them have been shown to have mycolic acid cyclopropanating activity when introduced into *M. smegmatis*, do not produce cyclopropanated mycolic acids [31,53-54]. The biochemical and the macro molecular differences between the pathogenic and non-pathogenic mycobacterium create a major challenge for taking forward of non-pathogenic hits to the pathogenic test. All evidences, therefore, clearly indicate that compounds which show activity in the *M.smegmatis* may not necessarily work on *M.tb*. The interaction between macrophage and *M.Tb*. is a critical step in the establishment of an early chronic infection. Because of these obvious differences, there is a dire need to confirm the activity of the compounds in pathogenic *M.tb in vitro* using cell based assay such as Monocyte Infection Assay (MIA). MIA is a quick and very established assay model to understand infection mechanism *in vitro* by *M.tb*. Monocytes is a host of both HIV and Mycobacterium and it has been reported that after infection with *M.tb*, monocytes enhanced HIV-1 replication[55,56]. Asymptomatic mycobacterium infection can lead to the disease syndrome under immunodeficiency condition followed by HIV infection. Therefore, hits established in this model can be extrapolated from the single mycobacterium infection to the co-infection model system.

Patient PBMC assay

Efficacy of hits against mycobacterium can be tested further in *ex vivo* before they go through the animal proof of concept. Peripheral Blood Mononuclear Cell (PBMC) isolated from tubercular patients can be considered as a very good *ex vivo* model particularly in a country where TB is widely prevalent. Test on collections of wide variety of patient samples in different stages of the infection with different strains of mycobacterium (MDR or XDR) will reveal the efficacy of the compound in diverse spectrum of disease situation. Several studies in animal models indicate that a series of immuno pathological event happen followed by mycobaterium infection. For example, infected cells from active TB patients showed significant production of nitric oxide as compared to that of uninfected cells[57-59] **.** Elevation in the level of IFN-gamma is also observed in human PBMC infected with *M.Tb.* A gross downregulation of gene expression associated with innate and adaptive immunity are observed in animal model infected with *M.Tb*. A lower relative expression of key innate immunity related genes including the Toll-like receptor (TLR2,4) genes, lack of differential expression of

indicator adaptive immune gene transcripts (IFNG, IL2, IL4) and lower major histocompatibility complex class I (BOLA) and class II (BOLA-DRA) gene expression was consistent with innate immune gene repression in the BTB-infected animals[58]. This wide array of differential gene expression pattern will certainly influence the effect of the functionality of drugs in PBMC isolated from patients in comparison to the normal human counterpart. Therefore, hits tested on MIA followed by the patient PBMC assay before exploring the animal model system will be more informative and cost-effective.

Animal efficacy model

The murine model has been considered as a central tool for the elucidation of protective immune mechanisms that are essential for controlling *M. Tb* infection. Additionally, the study of inbred mice has revealed significant divergence in the susceptibility and disease progression of individual mouse strains to an infection with *M. Tb*[60-61]. The continued study of genetically disparate mouse strains has the potential to identify immune mechanisms that correlate with increasing susceptibility to TB. These mechanisms will be highly applicable to studies in men and will assist in the early detection of individuals that are more vulnerable to the development of reactivation of TB. Also murine models of TB have the advantages of low cost, availability of immunological reagents, and the choice of inbred populations with varying susceptibility to aerosol infection[62-64]. Murine models for TB give the power to detect the differences in duration of therapies. Therefore, considering all those facts, mouse remains the most popular *in vivo* model for testing small molecules for their potential.

DRUG DELIVERY

Research on drug delivery is mainly driven by the two major factors, one is the targeted delivery where drug will be navigated to the diseased tissue and second is sustained release formulation where drug will be released in a controlled fashion. An ideal TB drug formulation should satisfy both of those parameters so that bioavailability of the drug to the targeted tissue in one hand is upregulated and controlled and on the other hand uniform release of the drug will reduce the chance of developing MDR and XDR-TB cases. Liposome encapsulated drug delivery is one of the recent attempts to increase the bioavailability and tissue specific distribution of TB drugs. Single intravenous dose of modified liposomes, loaded with rifampicin and isoniazid, targeted to deliver drugs in alveoli, maintain the plasma/tissue drug level for five-seven days. A significant reduction in bacterial count was reported when mice were going through a weekly dose for six consecutive weeks in comparison to the same dose of free drug treatment. Chemotherapeutic activity against murine TB using once weekly administered drugs such as, isoniazid and rifampicin, encapsulated in liposomes, augment several fold the antibacterial activity of those two drugs[65]. Recent evidence shows that nanoparticle based drug delivery will replace all those existing methods because of high carrier capacity, stability, independence on the route of administration and feasibility of restricted release form the matrix. Frontline TB drugs like rifampicin, isoniazid and pyrazinamide show a significant effect when encapsulated with nanoparticles[66-68].

POST GENOMIC ERA AND SYSTEMS BIOLOGY IN DRUG DISCOVERY

Unveiling genome sequence of *M.tb* H37Rv in 1998 opens altogether a completely new horizon in the field of drug discovery. Though the bacilli harbour an estimated 4000 genes, about 52% of the predicted proteins have known functions and 376 putative proteins can be considered as unique to the organism because there is no apparent sequence homology with the existing data bases[69] . With the help of functional genomics, if some of them are detected as essential for the survival, then those macromolecules can be considered as a novel drug target for *M.tb*. Since the complete genetic information is also available for the non-pathogenic strains like *M.smegmatis* therefore, comparative genomics will provide a new outlook on the differential gene expression patterns across the strains. Comparison of gene expression arrays among the drug sensitive and drug resistance organism would reveal a significant clue regarding macromolecules responsible for the phenotype of

the organism and thus can generate new targets for anti-TB drug discovery[70-72]. Databases like GenoMycDB, comprise pair-wise sequence alignments of the protein coding sequences collected from five different pathogenic mycobacterium. A comparison of such data bases would be an added advantage to identify pathogen specific genes and can be used as a drug target for any particular class of mycobacterium[73] . Identification of genes supports the latency of the organism and can also have a significant implication in TB drug development. Recent studies on genes related to the latency of the organism identified isocitrate lyase and gene regulator ó-factor (SigF). Another study on amphibian TB model shows that genes with polymorphic PE-PGRS repeats are related to the granuloma formation, a stage associated with the chronic mycobacterium infection. Genes with the tandem PE-PGRS repeat encode a set of unique, exceptionally glycine and alanine rich protein located mostly on the surface of the mycobacterium and thought to be responsible for the pathogenesis. However, vaccination using those proteins failed to protect mice against TB infection most likely indicating the antigenic variation of PE-PGRS repeat genes[74]. Therefore, pathogenicity of mycobacterium does not solely depend on the regulation of a particular class of gene expression rather it may be a cumulative effect of several classes of stage specific gene expressions and thus justifies a highthoroughput global gene search to identify novel genes related to determine virulency and latency of the organism.

Recent introduction of systems biology in the field of drug discovery created a new niche to understand the overall regulatory network at the level of cells, tissues, organs and the organism. This emerging field has the potential to generate a specific molecular model as a target. Based on the computational modeling and predictive simulation, it will be very effective for identifying the control point of a molecular network which can be targeted for the drug discovery[75-77] . Integration of genome wide data on several molecules like mRNA, proteins and metabolites will provide a logical picture of their cellular behaviour. *M.tb* transcriptome complies the information of global analysis of gene expression under different conditions which would enable us to identify expression of stage specific gene and the products of those differentially expressed genes can also be considered as a subject of drug targets. Protein-protein interaction network, interactome, encompasses a variety of interaction like physical association of two proteins critical for the metabolic pathway or interaction needed to make biologically active complex. After analysis of the interaction network, Raman K *et al*[78] identified several critical drug targetable protein-protein interactions based on novel algorithm. One of the classical examples is the interaction of proteins drive the mycolic acid biosynthetic pathway. Presence of mycolic acid, arabinogalactan-mycolate covalently linked with peptidoglycan and trehalose dimycolate in the cell wall provides an extra protection to this organism to survive in the hostile phagolysosomal environment within macrophage, antibiotic treatment as well as help them to evade the host immune system. In silico analysis based on the recent biochemical as well as genetic information identifies InhA, AccD3, Fas, FabH, Pks13, DesA1/2, and DesA3 proteins are competent for the drug targets and it was also reported that FabH-InhA interaction is critical in the FAS-II biosynthetic pathway. A highthoroughput co-immunoprecipitation assay or Yeast-two-hybrid based assay, such as FRET assay can be very effective model for target assisted drug discovery. Therefore, system analysis of interaction network and global analysis of comparative gene expression using omics (Transcriptome, Reactome, Interactome) can highlight potential novel targets in TB drug discovery[79].

CONCLUSION

Development of new drugs in the field of TB continues to be more challenging because of the emergence of drug resistance organisms as well the cases of the opportunistic mycobacterium infection in HIV patients. On the other hand, crystal structures of proteins or enzymes, unrevealing mycobacterium genome sequence as well as systems biology have opened a new horizon in the field of drug discovery. Researchers, at this cross road, are facing an additional challenge in the field of the cost of new drug discovery, finding new therapy cheaper

than the existing treatment regimen and developing any new drug which will significantly reduce the treatment time. On the other hand, the molecular basis of drug discovery in the field of TB is very much in the rudimentary stage. In this review, focus was given on the molecular basis of drug discovery based on the recent information available in the field of molecular, cell and systems biology in the field of TB. As an example, a unique model of drug discovery based on protein-protein interaction was proposed where interacting proteins as well as the interaction is playing a critical role in the synthesis of cell surface molecule, mycolic acid, required for the pathogenesis of this organism. Target selection, conceptualized on the recent systems biology information, includes docking on unique enzymes related to pathogenicity such as cyclopropane synythetase, would be another potential model in the field of anti-TB drug discovery.

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Original Article

A STUDY OF THE SOCIO-DEMOGRAPHIC PROFILE AND TREATMENT OUTCOME OF PAEDIATRIC TUBERCULOSIS PATIENTS IN BANGALORE MAHANAGAR PALIKE AREA

Maria Nelliyanil[1]*, M.P. Sharada[2], Nitin Joseph[3]***, Shashidhar S Basagoudar[1]****, S. Jayaram[2]* and Dipak C Patil[1]***

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Summary

***Background*:** Tuberculosis (TB) continues to be one of the most devastating and widespread infections in the world. Of the nine million annual tuberculosis cases, about one million (11%) occur in children (under 15 years of age). Childhood tuberculosis is a neglected aspect of the tuberculosis epidemic.

***Objectives*:** To know the socio-demographic profile, type of tuberculosis and treatment outcome in paediatric tuberculosis patients

***Methodology*:**The study was conducted in nine Tuberculosis units of Bangalore city from January 2009 to December 2009. Five Tuberculosis units from the nine tuberculosis units were selected by simple random sampling, paediatric patients diagnosed as having TB and registered under RNTCP were included in the study till the sample size of 209 was reached. Data regarding socio-demographic profile and type of TB was collected and the patients were followed up to assess treatment outcome.

***Results*:** Most of the patients coming to the RNTCP centres belong to the under-privileged group. Most of the patients were in the age group of 1 to <6 years, (37.7 %), male to female ratio was observed to be 0.6:1. Majority of the patients lived in nuclear families (73.2%), belonged to low socio-economic status (95.5%) and dwelled in overcrowded houses (89.5%). 23% reported history of contact with tuberculosis patients. More than half of the patients (57.4%) were undernurished. In the study, 56.5% had pulmonary TB and 43.5 % had extra-pulmonary TB. 94.7% of the patients completed treatment.

***Conclusion*:**Paediatric tuberculosis still continues to be a major problem in one-five years of age who are undernourished and belonging to low socio-economic status. **[*Indian J Tuberc 2012; 59:* 207-213]**

Key words: Paediatric Tuberculosis, Socio-demographic profile, Treatment outcome.

INTRODUCTION

TB continues to be one of the most devastating and widespread infections in the world. It is estimated that one third of the world's population is infected with *Mycobacterium tuberculosis* and that each year about nine million people develop TB, of whom about two million die. Of the nine million annual TB cases, about one million (11%) occur in children (under 15 years of age). In countries worldwide, the reported percentage of all TB cases occurring in children varies from 3% to more than 25%.[1] TB is an important cause of morbidity and mortality in children worldwide, especially in resource poor countries. Children are most likely to develop disease after infection and are significantly more likely to develop extra-pulmonary and severe disseminated disease than adults. Infected children represent the pool from which a large proportion of future cases of adult TB will arise. In addition, childhood TB is a sentinel event, indicating on-going transmission of TB within communities.[2] Though an estimated one million new cases of TB occur in children worldwide each year, paediatric TB has not been given the same priority as its adult counterpart. Childhood TB is a neglected aspect of the TB epidemic. This "orphan disease "exists in the shadow

1. Assistant Professor 2. Professor and Head 3. Associate Professor
* Department of Community Medicine, A. J. Institute of Medical Sciences, Mangalore
** Department of Community Medicine, Mahatma Gandhi Medical College and Research institute
*** Department of Community Medicine, Kasturba Medical College, Manipal University, Mangalore
**** Department of Community Medicine, Raichur Institute of Medical Sciences
***Correspondence*:** Dr Maria Nelliyanil, Assistant Professor, Department of Community Medicine, A. J. Institute of Medical Sciences, N.H. 17, Kuntikana, Mangalore - 04; Phone: 9481915959; drmaria03@gmail.com

of adult TB and is a significant child health problem, but is neglected because cases are usually smear-negative and is thus considered to make a relatively minor contribution to the spread of TB.[3]

However, children are particularly vulnerable to severe disease and death following infection, and those with latent infection become the reservoir for future transmission following disease reactivation in adulthood, fuelling future epidemics. TB has always been given priority in India, but the interest in childhood TB has only been kindled only recently.

Studies of pediatric TB are scantily available both in global and national contexts. Reliable data on the burden of all forms of TB amongst children in India are not available. Hence, a study of pediatric TB cases was carried out to determine the socio-demographic profile, type of TB and treatment outcome in the TB units of Bruhat Bangalore Mahanagar Palike area, knowing that the socio-demographic profile can help in understanding the groups that are vulnerable to the disease and treatment outcomes will help us to know whether RNTCP regimen is effective in paediatric TB.

METHODOLOGY

A longitudinal study was conducted in nine TB Units of Bangalore city from January 2009 to December 2009.All paediatric patients in the age group of 0 to 14 years diagnosed as TB and registered under RNTCP were included in the study. A sample size of 201 was calculated using prevalence of extra-pulmonary TB of 47% [4] with 90% power. To obtain the calculated sample size, five TB units from the nine TB units were selected by simple random sampling. A total of 209 cases in these TB units were enrolled. Data collection was started after obtaining the institutional ethical committee clearance and permission from respective authorities from Bangalore Mahanagar Palike and health centre. Informed consent was obtained from the parents/or guardians. The data regarding socio-demographic profile, history of contact with TB patients, etc. was collected by a pre-tested questionnaire during their visit to the hospital / health centre and the patients were examined to assess the response to treatment and outcome of treatment. The data was analysed using epiinfoversion 3.4.1.Descriptive statistics (means, proportions, percentages), chi square test and Fischer's exact test was used.

RESULTS

Table 1 shows the age distribution of the patients, most belonging to one-five year group. The mean age of the patients in our study was 7.5 years. Table 2 shows the socio-demographic characteristics with overall more females (62.68 %) than males (37.32 %), male to female ratio was observed to be 0.6:1 but it was not statistically significant(P value >0.05). Majority were Hindus (58.4%) by religion and belonged to upper lower class(65.6%)according to modified Kuppuswamy's classification. It was observed that the percentage of pediatric TB cases progressively reduced with increasing educational status of their mothers. 54.1% of the mothers of the

Table 1: Distribution of study population according to age and sex

Age group in years	Male (%)	Female (%)	Total (%)
<1	2(67%)	1(23%)	3 (1.4%)
1-<6	32(41%)	47(59%)	79 (37.7%)
6-<10	21(34%)	41(66%)	62 (29.6%)
10-<15	23(35%)	42(65%)	65 (31.1%)
Total	78 (37.32 %)	131 (62.68 %)	209 (100%)

patients were illiterate, the highest level of education was intermediate or post high school diploma (1.9%).

Twenty three per cent of the patients gave a history of contact with TB patients and it was observed to be significantly associated with the type of TB (Table 3). In all, 87.5% of the contacts reported to have had sputum positive TB. About 89.5% lived in overcrowded houses (Overcrowding being defined using number of persons per room) and nearly 85% of them gave a history of being exposed to indoor air pollution. More than half of the patients were undernourished, of which 54.2% suffered grade I under-nutrition, 39.2% grade II, 5.8% grade III and 0.8% grade IV according to IAP classification.

BCG scar was present in 159 patients (76%) and there was a statistically significant association between presence of BCG scar and type of TB (Table 3). Pulmonary TB was reported in 56.5% of

Table 2: Socio-demographic profile of the patients

Character	**Number**	**Percentage**
Mean age	7.5	
Median age	8	
Mode	5	
Religion		
Hindus	122	58.4
Muslims	76	36.4
Christians	11	5.3
Type of family		
Nuclear	153	73.2
Joint	45	21.5
Three generation	10	4.8
Others	1	0.5
Socio-economic Status (Modified Kuppuswamy classification)		
Upper middle	9	4.8
Lower middle	61	29.9
Upper lower	138	65.6
Lower	1	0.5
Total	209	100

Table 3: Various determinants in pulmonary and extra-pulmonary tuberculosis

Determinants		**Pulmonary (N=118)**	**Extra-pulmonary (N=91)**	χ^2	**P value**
Sex	Male	47	31	0.7	>0.05
	Female	71	60		
Religion	Hindu	64	58	1.9	>0.05
	Muslim	47	29		
	Christian	7	4		
History of contact	Yes	35	13	6.865	<0.05
	No	83	78		
BCG scar	Present	99	61	8.412	<0.05
	Absent	19	30		
Overcrowding	Present	105	82	0.069	>0.05
	Absent	13	9		
Indoor air pollution	Present	100	79	0.179	>0.05
	Absent	18	12		

Table 4: Distribution of the study population having extra-pulmonary tuberculosis

Type of extra-pulmonary tuberculosis	Male	Female	Total (%) N=91
Cervical lymphadenitis	12(26%)	34(74%)	46 (50.7 %)
Tubercular Meningitis	8(38%)	13(62%)	21 (23.2 %)
Pleura	5(50%)	5(50%)	10 (11 %)
Abdominal tuberculosis	2(33%)	4(67%)	6 (6.5 %)
Generalised lymphadenopathy	1(50%)	1(50%)	2 (2.1%)
Spinal tuberculosis	1(50%)	1(50%)	2 (2.1%)
Inguinal lymphadenitis	1(100%)	0(0%)	1 (1.1 %)
Skin	1(100%)	0(0%)	1 (1.1 %)
Axillary lymphadenitis	0(0%)	1(100%)	1 (1.1 %)
Eye	0(0%)	1(100%)	1 (1.1 %)
Total	31(34%)	60(66%)	91 (100%)

Note:91(43.5%) cases suffered from extrapulmonary tuberculosis.

Table 5: Distribution of patients according to treatment outcome and type of TB

Treatment outcome	Pulmonary TB (%)	Extra Pulmonary TB (%)	Total (%) **N=207**
Treatment completed and cured	118(57.3)	88(42.7)	206(95.5)
Death	1(100)	0(0)	1(0.5)
Total	119(57.5)	88(42.5)	207(100)

(Fischer's exact test p=1)

Note:Of the total 209 patients enrolled in the study the treatment outcome could be obtained only for 207 patients as two patients had spinal TB and their treatment was extended beyond the study duration.

the patients, of which 10(18%) were sputum positive cases and remaining were sputum negative.

Table 4 shows the different types of extra-pulmonary TB, tubercular lymphadenopathy (55%) is the most common manifestation of extra-pulmonary TB followed by tubercular meningitis and among the tubercular lymphadenopathy, the cervical lymph nodes were the most commonly involved .Prevalence of HIV infection in the patients was 0.4%(one patient). 50.2% of the patients were put on category III, 44.5% on category I and 5.3% on category II. The mean weight gain at the end of treatment was 2.5 kg (SD=2). Table 5 shows the patient outcome of treatment in various categories of treatment. Overall treatment completion rate, cure rate and death rate was 94.7%, 4.8% and 0.5% respectively. RNTCP treatment regimen was shown to be effective in tubercular meningitis, a severe form of TB, 85.7% of the TB meningitis patients recovered and had no sequelae while 14.3% had sequelae in the form of motor deficit.

DISCUSSION

Our study revealed that the maximum number of patients were in the age group of one to five years, followed by the age group of 11 to 14 years. Similar findings were reported in a hospital based study done by Sushama Bai S *et al*,[5] in 1998 in Kottayam district of Kerala where they also observed that the maximum number of cases were in the one to five years' age group which was 49.5%. Our finding that there were more females (62.68%) is similar to the observation of Sharma S *et al*[6], a retrospective analysis of paediatric patients.

Our observation of history of contact with TB patients is similar to the findings observed by Madhi F *et al*[7] in a Paris suburb, where 22% had history of contact with TB patients. Among the children studied, 76.5% had a BCG scar which is similar to the findings of a retrospective study by Sivanandan S *et al*[8] and Gupta R *et al*.[9] The prevalence of undernutrition observed by us is a little higher (57.4%) than that observed by Sushmabhai S *et al*[5], they had a prevalence of 42%. The proportion of extra-pulmonary cases observed by us is similar to that observed in a retrospective analysis of pediatric TB cases carried out at the LRS institute of TB and Respiratory Diseases, New Delhi (Arora V K *et al*[6]) which reported that 47% of the cases were extra-pulmonary TB. Among the extra-pulmonary TB, tubercular lymphadenopathy is the most common manifestation followed by tubercular meningitis (23.2%), pleural effusion (11%), abdominal TB (6.5%), spinal TB (2.1%), skin and eye TB (1.1%) each. A hospital-based study in paediatrics patients with extra-pulmonary TB by H C Maltezou *et al*[10] between 1982 and 1998, also showed that lymphadenitis (47%) was the most common manifestation of extra-pulmonary TB, followed by pleural effusion (26%), meningitis (16%),

skeletal TB (5%), miliary TB (3%), abdominal TB (2%), and pericarditis (1%). The difference in rates of types of extra-pulmonary TB may be because H C Maltezou's study, was a hospital-based study. In our study, there were no cases of miliary TB, as they are patients in a serious condition and would be hospitalised and wouldn't come to the RNTCP centres. Prevalence of HIV infection in the patients was 0.4% (one patient). Several studies have shown a prevalence between 0.8 to 2%.[11-13] This difference may be due to the fact that the above studies were done in hospitalised patients whereas our study was done at the DOTS centres. 50.2% of the patients were put on Category III, followed by 44.5% on Category I and 5.3% on Category II in the present study while in Kabra *et al*'s study of the total 459 patients,70.3% patients were in Category I, 2.6% were in Category II and 26.1% in Category III.[14] This difference may be explained by the fact that their study was hospital-based. However, Category III has been merged into Category I or called as the new case regimen now but when the study was done, Category III regimen was still in use. The mean weight gain at the end of treatment was 2.5 kg (SD=2),while a study on weight gain in patients with TB treated under directly observed treatment short-course in all age groups across all ages by M Vasantha, revealed mean change in weight of 3.2 kgs.[15]

We observed an overall treatment completion rate, cure rate and death rate was 94.7%, 4.8% and 0.5% respectively. According to RNTCP, cure is defined only for sputum positive pulmonary TB, since most of the paediatric patients have sputum negative TB and extra-pulmonary TB the outcome of treatment was declared as treatment completed. However, as the symptoms of the patients improved, they can be considered apparently cured. Thus the treatment outcome of paediatric patients put on RNTCP treatment regimen is in accordance with the objective of the programme. Sharma S *et al* in their study also observed that the treatment completion rate was 94.9% and death rate was 0.3%.[6] There was no significant difference in the treatment outcome based on gender or treatment outcome as was also observed by A D Harries *et al*.[16] 85.7% of the TB meningitis patients recovered with no sequelae,which is similar to the observation of a prospective study done by C K Indumathi *et al* to evaluate intermittent short course therapy for pediatric TB where 86 % children had complete cure without sequelae.[17]

CONCLUSION

This study has observed that paediatric TB still continues to be a major problem in one to five years of age who are undernourished and belonging to low socio-economic status. Poor housing conditions which continue to haunt our population is an important risk factor for TB transmission. Thus improving the socio-economic conditions and proper treatment of adult TB who are the sources of infection to children will go a long way in preventing paediatric TB and protect children who are the future of our country.The RNTCP DOTS strategy is an effective treatment modality for TB in children achieving a high treatment completion rate (94.7%), and low death rate (0.5%)

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Original Article

AGREEMENT BETWEEN SKIN TESTING AND QUANTIFERON®-TB GOLD IN-TUBE ASSAY (QFT-TB) IN DETECTING LATENT TUBERCULOSIS INFECTION AMONG HOUSEHOLD CONTACTS IN INDIA

Satyajit Pattnaik[1]*, K. R. John[2], E. Shalini[3]*** and J. S. Michael[4]*****

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Summary

Aims: The present study was designed to find the agreement between Tuberculin Skin Test and interferon gamma assay test in detecting latent tuberculosis infection in household contacts of sputum culture positive tuberculosis cases.

Setting: Department of Community Medicine, Christian Medical College, Vellore.

Methods: One hundred and fifty household contacts of sputum culture positive tuberculosis cases were tested with both the methods simultaneously and actual as well as kappa agreement was determined.

Results: The overall actual agreement between both the tests was found to be 82% with a kappa agreement of 0.57**.**

Conclusion: The agreement was very high (both percentage agreement and Kappa) in pediatric contacts but it was poor in adult contacts. **[*Indian J Tuberc 2012; 59:* 214-218]**

***Key words*:** QFT, MDR TB, Household contacts

INTRODUCTION

Tuberculosis (TB) remains a major public health problem. Out of estimated global annual incidence of 9.4 million TB cases, two million cases were estimated to have occurred in India.[1] TB spreads by inhalation of infected droplets. The risk of getting infected is more among household contacts and in crowded places, especially in high endemic countries.[2] Infected contacts will then develop a progressive immune response and some (between 5-10% of those who are exposed) will develop TB disease in their life span.[3] For nearly a century, the screening for latent TB infection relied upon Tuberculin Skin Testing (TST). While TST is a useful guide for identifying tuberculosis infection, it has a number of drawbacks.[4] The recent introduction of T-cell based interferon gamma assay like (QuantiFERON®-TB Gold In-Tube assay (QFT-TB) has demonstrated a role in screening for latent TB infection and contact tracing, and has reportedly overcome some of the drawbacks of TST.[5,6] None of these is a gold standard test for diagnosis of latent TB infection. Patients with multi drug resistant (MDR) TB are infectious for a long time and can spread the disease to contacts over an extended period. The present study was undertaken with the objectives of finding the agreement between the two tests (i.e., TST and QFT-TB) in detecting latent TB infection.

MATERIAL AND METHODS

Study population

This cross-sectional study was conducted among household contacts of sputum culture positive TB cases that were suspected MDR TB patients belonging to a population covered under Tuberculosis Unit (TU) of District Tuberculosis Centre, Vellore and another Tuberculosis Unit managed by the Community Health Department of Christian Medical College (CMC), Vellore. Among all suspected MDR cases in these two TUs, a total of 147 have done sputum culture testing. Out of 147 cases, 108 were found to be culture positive for *Mycobacterium tuberculosis*. Out of these 108 cases, 48 were selected on the basis of close proximity to

1. Assistant Professor 2. Professor & Head 3. Associate Research Officer 4. Professor

* Department of Community Medicine, Kalinga Institute of Medical Sciences (KIMS),Bhubaneswar

** Department of Community Medicine, SRM Medical College, Kancheepuram

*** Department of Microbiology, Christian Medical College, Vellore

***Correspondence*:** Dr. Satyajit Pattnaik, Assistant Professor, Department of Community Medicine, Kalinga Institute of Medical Sciences (KIMS), KIIT University, Bhubaneswar (Odisha); Email: drsatyajitpattnaik@gmail.com

the institution where the study was conducted. Out of these 48 cases, 34 agreed to participate in the study.

The study was approved by the Institutional Review Board and Ethics Committee of CMC, Vellore. 2 Tuberculin Unit (2TU) PPD RT 23 with Tween 80 obtained from Statens Serum Institute, Denmark was used for the Tuberculin Skin Testing (TST) after obtaining necessary permission from the Drugs Controller General of India. Two trained tester and reader were hired for the purpose. TST was done on the contacts. The TST was read after 48-72 hours.

Blood was collected from the contacts simultaneously for QFT-TB testing at the Microbiology Laboratory of CMC, Vellore.

QuantiFERON®-TB Gold In-tube assay

QFT was performed according to the manufacturer's (**Celletis Ltd., Victoria, Australia**) instructions. Blood samples were collected in three special tubes: one coated with the *Mycobacterium tuberculosis* (MTB)-specific peptides ESAT-6, CFP-10, and TB 7.7 (Rv2654, only peptide 4); one with mitogen as a positive control; and one without antigen as a negative control. The tubes were incubated for 24 h at 37°C, followed by centrifugation . The serum was separated and stored at -20ºC until testing. The concentration of IFN-ã in plasma was measured using a commercial QFT ELISA. The test result was determined as negative, intermediate, or positive (cut off at 0.35 IU/mL and ≥ 25% of the nil control) using the manufacturer's software.

Statistical analysis

A TST cut off of ≥ 10 millimeter and a QFT cut off of 3.5IU/ml were considered as positive. The percentage agreement (either positive or negative) between TST and QFT was estimated. Kappa, another measure of concordance which corrects for agreement expected by chance, was also estimated with its 95% confidence interval (95% CI), This agreement was further analyzed in pediatric and adult age groups.

RESULTS

The total number of contacts screened were 154. In these 154 cases, 150 valid results were obtained. The rest four could not be followed up.

Table 1a: Overall Agreement between TST and QFT

	QFT POSITIVE	QFT NEGATIVE	TOTAL
TST POSITIVE	94	26	120
TST NEGATIVE	1	29	30
TOTAL	95	55	150

Overall agreement is **82%** and Kappa is **0.57**(95% CI 0.434-0.706)

Table 1b: Overall Agreement between TST and QFT (Pediatric group)

	QFT POSITIVE	QFT NEGATIVE	TOTAL
TST POSITIVE	30	2	32
TST NEGATIVE	0	18	18
TOTAL	95	55	50

Overall agreement is **96%** and Kappa is **0.915** (95% CI: 0.917-1.033)

Out of 150 contacts, 50 (33.3%) were of pediatric age group (< 15 years) and rests were adults. Of all 150, 61 (40.7%) had BCG scar and 89 (59.3%) had no BCG scar.

The overall actual agreement between TST and QFT was 82.0% and the kappa agreement was 0.571(95% CI: 0.434-0.706) (Table1 a). Kappa agreement between TST and QFT was 0.915 (95%

Table 1c: Overall Agreement between TST and QFT (Adult group)

	QFT POSITIVE	QFT NEGATIVE	TOTAL
TST POSITIVE	64	24	88
TST NEGATIVE	1	11	12
TOTAL	65	35	100

Overall agreement is **75%** and Kappa is **0.352**(95% CI: 0.714-0.533)

Table 2: Agreement between QFT and TST in healthy population with varying risks for LTBI

Study/Year	Country	Risk Group	Total Participants	BCG Vaccinated (in %)	Actual Agreement (in %)
Brock *et al*., 2004	Denmark	Contacts of persons with TB	45	0	93
Pai *et al*., 2005	India	Health Care Workers	719	71	81
Kang *et al*., 2005	Korea	Close & casual contacts of persons with TB	120	73	53
Porsa *et al*., 2006	USA	Prisoners	409	-	89
Ferrara *et al*., 2006	Italy	Hospitalized adults	286	18	73
Harada *et al*., 2006	Japan	Health Care Workers	304	91	13
Dogra *et al*., 2006	India	Hospitalized children	97	82	94
Mahomed 2006	South Africa	Healthy adults	358	81	68
Tsiouris *et al*., 2006	South Africa	Pediatric contacts	184	73	78
Lee *et al*., 2006	Korea	Healthy students	131	100	74
Nakaoka *et al*., 2006	Australia	Pediatric contacts	75	49	81
Connel *et al*., 2006	Australia	Pediatric contact	75	49	64

CI: 0.797-1.033) in pediatric contacts (Table 1 b) as compared to 0.352 (95% CI: 0.714-0.533) in adult patients (Table 1 c).

DISCUSSION

In regions with a high incidence of TB, a search for contacts among the relatives/house hold members of smear positive cases may succeed in detecting a large number of secondary cases.[7] Soon after the introduction of antibiotics (as early as 1959), trials were conducted which demonstrated that, properly prescribed and taken, preventive treatment reduces the risk of future disease and is cost-effective.[8] As individuals with latent infection with TB by definition are healthy and do not have radiological abnormalities (except a few), screening for detecting infection must rely on its immunological markers.

While TST is a useful guide for identifying tuberculosis infection, it has a number of drawbacks including the need for a repeat visit to read the test, problems in interpretation due to cross-reactivity with other mycobacterial species, the booster effect, and false negative results because of intercurrent immunosupression, as well as the variability inherent in its application and reading.[4]

Many studies have compared the agreement between whole blood interferon gamma (QFT) assay with TST in different countries (Table 2). The percentage agreement found between both tests for all contacts in our study (i.e., 82%) is consistent with the findings from majority of the studies in the endemic countries. The study[8] by Pai *et al* among health care workers in India, had reported an agreement of 81.4% (Table 2). Percentage agreement suffers from serious disadvantage of an indicator of agreement. There can be considerable agreement by chance alone even if the two methods have different criteria for measurement. One way to overcome this problem would be by using Kappa statistics which corrects for chance agreement. The Kappa agreement found between both tests for all contacts was only 0.57 (moderate agreement). It could be observed that among the 150 contacts, 120 (80%) were TST positive compared to 95 (63.3%) and the difference in the concordance cells was statistically significant by Mc Nemar's Chi-square test. This showed that the two methods are not similar in detecting latent TB infection. This could be due to mix-up of both children and adults for comparing the infection. The TB infection among adults is much higher (Male-66%, Female-57%)[9] compared to children and most of them would have been infected much earlier.

The percentage agreement between TST and QFT was found to be high (96.0%) in pediatric age group in the present study which is consistent with other studies in pediatric age group (Table 2)[8]. The Kappa agreement was 0.951 (95% CI: 0.797-1.033) which showed that the strength of agreement was almost perfect. This is true because children are considered to be the ideal population to find the extent of TB infection in the community.

Our study showed that in BCG vaccinated individuals, the percentage agreement is 90% whereas in BCG non-vaccinated, it is 76%. The Kappa agreement was 0.779 and 0.411 respectively (not tabulated). These results may not be comparable as a proportion of the persons vaccinated at birth do not leave behind a scar and in another proportion the BCG scar wanes in due course. In a study among health care workers in India, Pai *et al* observed that a history of BCG vaccination had little impact on TB infection.[10]

Tuberculin survey[11] conducted in south zone of India as part of the nation-wide survey demonstrated that infection among BCG vaccinated and not-vaccinated children were similar. The limitations of study also need to be considered while interpreting the results.

The study included 150 household contacts (50 children and 100 adults) which was inadequate to obtain the results with required precision. The sample of contacts in our study was selected for operational convenience and close proximity to the institution.

CONCLUSION

The study showed a good agreement between TST and QFT in detecting latent TB

infection among pediatric group of children. This finding is consistent with other such studies. Thus, the decision to use any one of them will depend on the setting in which it is used and the resources available. The study also warrants for a larger study including more participants.

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Original Article

EQUIVALENCE OF ACID ALONE OR ACID-ALCOHOL AS DECOLOURIZING AGENT IN ZIEHL - NEELSEN METHOD

M. Gomathi Sekar, Fathima Rehman, Vanaja Kumar and N. Selvakumar

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Summary

***Background*:** Microscopists opine that acid-alcohol decolourized slides may enhance acid-fast bacilli (AFB) smear positivity, and published documents on equivalence of acid and acid-alcohol in ZN staining method are not easily accessible.

***Setting*:** National Institute for Research in Tuberculosis, Chennai, India.

***Objective*:** To document the equivalence of 25% sulphuric acid (ZN-acid method) and 3% hydrochloric acid-alcohol (ZN-alcohol method) as decolourizing agents in ZN method for detection of acid-fast bacilli.

***Methods*:** Two smears from each of 253 sputum samples from pulmonary tuberculosis patients, prepared and allocated, one to ZN-acid method and another to ZN-alcohol method were read blind. All the specimens were cultured for *Mycobacterium tuberculosis* by modified Petroff's method. Culture of *M. tuberculosis* was gold standard.

***Results*:** The concordance between the methods was 85% (kappa 0.68), and the sensitivity (79%) and specificity (89%) were same for both the methods.

***Conclusion*:** In conclusion, the common belief that acid-alcohol decolourized slides give enhanced smear positivity stands void. **[*Indian J Tuberc 2012; 59:* 219-223]**

***Key words*:** AFB, Decolourizing agent, *Mycobacterium tuberculosis,* Ziehl-Neelsen

INTRODUCTION

Detection of acid fast bacilli (AFB) in sputum or any biological sample is diagnostic of tuberculosis[1]. It is achieved generally by staining the smears by hot Ziehl-Neelsen (ZN) method all over the world. ZN method generally involves staining, decolourizing and counter-staining the smears, respectively, by basic fuchsin solution, dilute acids or acid-alcohol solution and methylene blue solution to achieve best results[2]. However, apprehension persists in the field on the recommended use of 25% sulphuric acid over 3% acid-alcohol as decolourising agent in ZN method (non-documented feedback from the field). The apprehension is that decolourization with acid presumably gives unclean smears for examination under the oil immersion microscopes and reduces AFB smear positivity rate. It is also opined that acid-alcohol decolourized slide gives clean smears and enhances the smear positivity. In addition, lack of accessible documented evidences on the equivalence of acid and acid-alcohol in the ZN method[3], especially in this era of information technology, and recent scientific interest in the investigation of efficiency of dilute hydrochloric acid as a decolourizing agent in the ZN method[4], prompted us to document our observations on the equivalence of acid and acid-alcohol as decolourizing agents in ZN method.

MATERIAL AND METHODS

Consecutive two hundred and fifty three sputum samples from pulmonary tuberculosis suspects, assessed for admission into the controlled clinical trials at National Institute for Research in Tuberculosis (NIRT), were selected. Of these, 33 were diagnostic samples collected before initiating treatment, 94 were from patients during treatment or follow up period and the remaining 126 were from patients who were assessed for admission into clinical trials but not included in clinical trails for different reasons including the previous history of treatment. Patient's consent was obtained before admitting them in controlled clinical trials and the Institutional ethics committee's clearance was obtained. From each of the samples, two direct smears were made and heat fixed on hot plate. One

National Institute for Research in Tuberculosis (ICMR), Chennai (Tamil Nadu)

***Correspondence*:** Dr. N. Selvakumar, Scientist-'G' and Head, Department of Bacteriology (Retd), National Institute for Research in Tuberculosis (ICMR), Chetput, Chennai - 600 031 (Tamil Nadu); Email: selvakumar.nagamiah@gmail.com; Fax No: +91-(044)-28362528/2525

smear was allotted to hot ZN method using 3% hydrochloric acid-alcohol (ZN-acid alcohol method) as a decolourizing agent and other to ZN method using 25% sulphuric acid (ZN-acid method). All the smears were coded and read blind. For the ZN-acid method, the preparation of direct smears and reagents, and staining, examination and grading protocols were followed as per RNTCP laboratory manual.[5] All positives and 20% of negative slides were checked by a senior technician. Referee reading resolved any discrepancy in smear results. The results of the referee reading were taken as final. The smear results were decoded and used for analysis. All the sputum samples were processed by modified Petroff's method and cultured on solid Lowenstein Jensen (LJ) medium. The *M. tuberculosis* isolated were identified by phenotypic methods followed in NIRT.[6] The sensitivity and specificity were calculated against culture of *M. tuberculosis* as the gold standard. The agreement between ZN-acid and ZN-acid alcohol methods was studied using kappa statistics.

PREPARATION OF THE REAGENT

Acid alcohol (3%): To 485 ml of alcohol (Tamil Nadu State Government supply) in a flask, 15 ml of concentrated hydrochloric acid (Qualigens, India) was added.[1]

Sulphuric acid (25%): To 375 ml of distilled water, 125 ml of concentrated sulphuric acid (Qualigens, India) was added keeping the flask in cold water. Two batches of reagents were prepared and used. The other reagents, 1% carbol fuchsin and 0.1% methylene blue were prepared as per RNTCP guidelines.[4]

RESULTS

For all the samples studied, agreement of smear results between ZN-acid and ZN-acid alcohol method was 85% (*k value* = 0.68) (Table 1). Out of 253 sputum samples, 11 were eliminated; three as

Table 1: Smear results obtained from ZN method using 25% sulphuric acid (ZN-acid) and 3% acid alcohol (ZN-alcohol)

		ZN -acid						
		Scanty*	**1+**	**2+**	**3+**	**All positives**	**Negative**	**Total**
ZN - alcohol	**Scanty**	2	3	0	0	5	9	14
	1+	4	19	8	5	36	7	43
	2+	0	4	3	4	11	1	12
	3+	0	1	6	16	23	1	24
	All positives	6	27	17	25	**75**	**18**	93
	Negative	11	5	0	0	**16**	**144**	160
	Total	17	32	17	25	91	162	253

* 3 +: More than 10 AFB per oil immersion field in at least 20 fields; 2 +: 1–9 AFB per oil immersion field in at least 50 fields; 1+: 10–99 AFB in 100 oil immersion fields; Scanty: 1–9 AFB in 100 oil immersion fields.

contaminants and eight as non-tuberculous mycobacteria (NTM). Out of three contaminated samples, one each was positive in ZN-acid and ZN-acid alcohol method. Among the eight samples that yielded NTM, two were smear positive in ZN-acid alcohol method only. Of the remaining 242 samples which were analysed, 91 were culture positives. The sensitivity and specificity were 79% and 89% respectively either for ZN-acid or for ZN-acid alcohol method (Table 2). In this study, 17 culture negative samples (15 from follow-up /

Table 2: Comparison of smear results obtained from ZN using 25% sulphuric acid (ZN-acid) and ZN using 3% acid alcohol (ZN-alcohol) with culture results

	Smear results**	**Culture results***						
		Cols	**1+**	**2+**	**3+**	**All positive**	**Negative**	**Total**
ZN - acid	**Scanty**	0	3	6	1	10	6	16
	1+	2	9	4	8	23	9	32
	2+	1	1	7	7	16	1	17
	3+	0	1	2	21	24	1	25
	All positives	3	14	19	37	**73**	**17**	90
	Negative	7	7	4	1	**19**	**133**	152
	Total	10	21	23	38	92	150	242
ZN-alcohol	**Scanty**	2	1	2	0	5	8	13
	1+	3	8	10	12	33	8	41
	2+	0	2	3	7	12	0	12
	3+	0	0	5	18	23	1	24
	All positives	5	11	20	37	**73**	**17**	90
	Negative	5	10	3	1	**19**	**133**	152
	Total	10	21	23	38	92	150	242

*3+: confluent growth; 2+: innumerable number of colonies; 1+: >20 to 100 colonies; Cols: 1–19 colonies; all positive: total positives; Negative: no growth of *M. tuberculosis*;

**** As shown in the previous table**

assessment patients and two from new cases) were smear positive in either of the methods.

DISCUSSION

The higher smear positivity rate (79.3%; 73/92) observed in this study could be due to the selective referral of pulmonary tuberculosis suspects from the peripheral clinics. The reduced specificity (89%; 133/150) could be attributed to the inclusion of samples collected during treatment and follow up period. It is known that 'smear positive and culture negative' samples could range from 20-25% among samples collected and studied during treatment [7] and in this study 15 of 17 'smear positive and culture negative' samples were from patients on treatment/ follow up.

National Tuberculosis Programmes (NTP) recommend the use of 25% sulphuric acid as decolourizing agent in hot ZN method although WHO and UNION (International Union Against Tuberculosis and Lung Disease) recommend either 25% sulphuric acid or 3% acid-alcohol [1, 2]. NTPs prefer the use of acid, as procurement and preparation of 25% sulphuric acid is less cumbersome for the district TB programme officials compared to the lengthy regulated administrative procedures needed to procure alcohol, and avoid stock outs in their stores. In addition, the stringent storage conditions and safety precautions to prevent theft in the health facility are added risks to the use of alcohol in peripheral health settings.[8]

The anticipated advantage of using acid-alcohol in ZN method could be that *M. smegmatis* can be decolourised with alcohol in biological samples, especially in urine samples.[9] Therefore, the diagnosis of renal tuberculosis in urine samples should be unequivocally confirmed with ZN-acid alcohol method as *M. smegmatis,* excreted in urine as result of transient colonization in urinary tract, will not be stained and mis-diagnosed for tuberculosis. In India, where the causative organism of pulmonary and extra-pulmonary tuberculosis is predominantly *M. tuberculosis*, the use of 25% sulphuric acid in hot ZN method will meet all the requirements and the use of acid-alcohol in the ZN method is not warranted for sputum samples in the diagnosis of pulmonary tuberculosis. The present study also, though small in its magnitude, reveals that acid-alcohol didn't show any added advantages over sulphuric acid in the detection of AFB in sputum samples. Nevertheless, the quality of smears, especially the staining characteristics in both the methods, which was not documented in the study, might have given some inputs over cleanliness of smears.

CONCLUSION

In conclusion, the findings reveal that 25% sulphuric acid is as good as 3% hydrochloric acid-alcohol as a decolouriser in ZN method for detection of AFB in sputum samples, and the common belief in the field that the acid-alcohol could yield enhanced smear positivity stands void.

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Original Article

PERSPECTIVE OF TUBERCULOSIS PATIENTS ON FAMILY SUPPORT AND CARE IN RURAL MAHARASHTRA

Aarti Kaulagekar-Nagarkar[1], Deepali Dhake[2] and Preeti Jha[2]

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Summary

Background: Role of patients' family in TB-control programme has received least attention in research and negligible attention is paid to support and care experiences of patients in rural societies.

Aim: Present study aims at collecting qualitative data on how tuberculosis patients define support and care during illness, and document their experiences and perspectives about care and support.

Methods: This is a qualitative study with grounded theory approach. Data were collected by conducting series of 15 focus group discussions (FGD) covering 113 patients from rural Pune district of Maharashtra. Thematic analysis was undertaken after preparing detailed transcripts of each FGD.

Results: Good support and care was considered as receiving necessary attention and help in daily routine, monitary help, emotional and moral support and motivation for early recovery. Family provided support by accompanying to the health centre, reminding about medicines, giving meals. Female patients reported less sympathetic attitude and unfair treatment at husband's home while males received emotional and physical support from spouse. Stigma led to discrimination and hindered the support and care mechanism.

Conclusion: Family awareness and preparedness for providing support need to be strengthened. Counselling and motivation during each visit are the keys to successful completion of treatment. There is need to make counsellors/psychologists available in the existing system. **[*Indian J Tuberc 2012; 59:* 224-230]**

Key words: Tuberculosis, Family support, Stigma, Rural, India

INTRODUCTION

Despite the availability of highly efficacious treatment for decades, India is the highest TB burden country accounting for one fifth (21%) of the global incidence.[1] Studies on care seeking behaviour of chest symptomatics[2-4] are ample in numbers but studies dealing with family support and care offered to tuberculosis patients are dismal. Moreover, in case of TB, a chain of events starting from exposure to infection, manifestation of symptoms, access to health facility, treatment and recuperation, family support and care play a pivotal role and decide outcome to an extent.[5] TB patients face various barriers in day-to-day life; so also isolation and rejection from families and communities.[6] Concerns and expectations of TB patients are required for improving quality of care, their compliance and completion of the treatment.[7] Family and society are the major constituents of social structure. Last two decades have witnessed the research on patients' health seeking behaviour which is influenced by gender, culture and family.[8-12] However, studies on patients' definition of support and care and their expectations from family are rarely documented. Objectives of this paper are to study perception about support and care of TB patients, to understand availability and kind of support received during illness, and to document their experiences and perspectives about care and support.

MATERIAL AND METHODS

The study was conducted in the rural areas of Pune district, located in the western part of Maharashtra, India. The Revised National Tuberculosis Control Programme (RNTCP) which follows the policy guidelines of the WHO's DOTS strategy, after its successful testing was launched in 1997.[13] Pune rural has eight TB units and 50 microscopy centres while the treatment recommended under RNTCP is

1. Assistant Professor 2. Research student
Interdisciplinary School of Health Sciences, University of Pune, Pune
Correspondence: Aarti Kaulagekar Nagarkar, Ph.D., Interdisciplinary School of Health Sciences, University of Pune, Ganesh Khind, Pune - 411007 (Maharashtra); Phone: 91-020-25691758 (O); 9822437743 (Mobile); Email: aarati@unipune.ac.in, amnagarkar@gmail.com

available at 105 primary units at village level. The current paper is based on the data collected from purposively selected five units which primarily covered Dehu, Fursungi, Urali, Khed and Khanapur villages and surrounding areas. These units were selected because of a sizeable number of patients registered for treatment and available for FGD.

Data collection tools and techniques

Participants in the study were tuberculosis patients. They were approached to explain the study when they visited DOTS centres for their regular treatment. The study included patients who were more than 18 years and less than 60 years of age. Those patients who were very sick were not included in the study. Patients were first assessed for their willingness to be a part of the study and availability for FGD. FGD was arranged only after enough number of patients agreed to participate. For those who showed willingness to participate, the details of the study were elaborated and explained to each one of them and written informed consent was obtained. Prior to the initiation of the study, study protocol including informed consent form and its translation in local language was scrutinized and approved by the University's Ethics Committee. Male and female patients, currently on anti-tuberculosis treatment, participated in the FGD. In each primary unit, three discussions (one male, one female and mixed group) were conducted. A total of fifteen FGDs were arranged comprising 6-12 tuberculosis patients in each FGD. Thus, the findings are based on the opinions expressed by more than 113 tuberculosis patients.

Description of study population

There were more males (n=58) than females(n=55) and nearly 35 per cent were between 18 to 30 years. There was almost equal distribution of young, middle aged and late middle aged patients. Majority of them were married (65.5%) and from extended family (63.7%) set up. Nearly forty per cent were illiterate and equal number had studied upto secondary school. Therefore, none of them were in any skilled job employment, majority were landless labourers (31.8%) and farmers (26.5%) while one fourth (24.7%) of them were unemployed.

Tools and techniques

FGD guide was developed based on the review of current literature and in the light of objectives of research. FGDs were arranged at familiar and convenient places for the patients, mostly community halls nearby a DOTS centre. Following questions were discussed in each session; what are the commonly seen symptoms of TB? What do you think causes TB? What are the changes observed in personal, family, and social life due to the disease? What was your own and family members' first reaction after diagnosis of disease? What do patients expect from family in terms of support and care? Can you share positive/negative experiences with regard to support and care? How do families provide day-to-day support and who is likely to get involved more in providing support? What other support and care does one expect from family? Was there a gap between expected care and actual experiences? FGD guide was pilot-tested prior to the study. A team of three researchers conducted the discussions. One was responsible for recording the information, making detailed notes and the second researcher was observing and noting down expressions, body language. A moderator handled the proceedings and ensured participation of all members. FGD session was concluded when intended information was obtained. The information collected was expanded in detailed notes on the same day and thematic analysis was carried out. Validity of these findings was augmented by presenting them to some of the participants during subsequent visits. Thus validated results are summarized here.

RESULTS

Results are organized as per the themes discussed during FGDs.

Awareness about TB symptoms

Reported chief symptom was persistent cough, fever and loss of appetite. Men mentioned cough with streak of blood in sputum while women mentioned breathlessness, general weakness along with weight loss and loss of appetite. Commonly reported cause of the disease was 'contact with a

tuberculosis person', known or unknown case. Inhalation of dust, exposure to cold weather while working on agricultural farms and prolonged and recurrent episodes of cough and cold progressed to tuberculosis were other causes reported. Respondents mentioned that men caught infection quickly when they came in contact with an infected person at the time of travelling in crowded buses during their visits to cities. Drinking alcohol, smoking and childhood asthma were other perceived causes of disease among men. Men in multiple relations (referred as 'baher, dusarikade janara') were more vulnerable to tuberculosis according to some of the respondents. Women participants felt that eating less food, hard agriculture labour, and black magic predisposed them to the disease.

Effect of TB diagnosis on personal and social life

Discussion began by eliciting their responses on perceived effect of TB diagnosis on their personal as well as social life. This question was asked to understand primary concerns of patients and reactions of immediate family and relatives. Concerns and reactions helped in identifying domains where support was anticipated and nature of the 'support and care' received.

Consequences on personal life

Participating patients mentioned leading tough life at personal as well social level. Female patients mentioned that husband and family often taunted them for contracting the disease. Younger children were prevented from interacting with such mothers and were given misleading information about mother's moral character and behaviour. Some opined that personal life of married women ruined and they were forced to live in a fear of abandonment or husband's remarriage. Female respondents were displeased with their family environment and often felt depressed because of the disease. Help from parents was very vital for both male and female patients. Almost all male respondents reported economic loss due to tuberculosis. Most of them were daily wagers hence worried about meeting daily needs. They mentioned about buying ration and provisions on credit or borrowing money. Some of them were of the opinion that government should provide soft loans to TB patients.

Consequences on social life

Respondents mentioned that community at large was scared of contracting the disease and therefore it was necessary to maintain distance from neighbours. They felt isolated, ostracized but did not blame community for such rude behaviour. Experience of stigma was reported by each and every member of these groups. Fear of rejection kept them away from participation in social events. Patients mentioned that isolation and discrimination was everyday experience; families discriminated subtly while community showed their rejection overtly.

First reaction after diagnosis

Participants were asked about family's first reaction upon learning about their diagnosis. All discussions yielded similar information, majority of the families could not believe the 'news', they were shocked, deeply saddened and in some cases denied the diagnosis. It appeared that the families were not prepared to receive such news. Later on, patients were asked to share their expectations from families immediately after diagnosis and current expectations till completion of their treatment. Emotional support and physical help provided by family at the beginning, that is immediately after diagnosis, was different from support and care received in day-to-day life. Participants were pleased with the support provided to them by their families during diagnosis. It was also stated that they received good emotional support and care from their families but it might not happen with all the patients and very few TB patients were lucky to get continued care from their family members.

Concept of Support and care

Their expectations can be categorized into two distinct terms, that is support *(madat)* and care *(kalaji)*. They were further asked to define these terms by citing examples. Good support and care was defined (by patients) as receiving necessary attention and help in daily routine, monitary help,

emotional and moral support and motivation for recovery. According to them, 'support' could be measured in terms of accompanying somebody for treatment, reminding about medicines, food and water served in the bed, allowing to take rest and all other care as and when demanded. While 'care' was described as speaking words of encouragement, motivate to fight with the disease, provide hope and positive thinking, discouragement from negative thoughts like suicide or running away from home.

Support and care provided by family

In order to extract information on actual role of family, participants were asked several questions. The selection of questions was dependent on the way earlier discussion had taken place. Questions used during FGDs are as follows:

a) All of us require help during illness, according to you, who should take care of TB patients and what is your experience about it? b) Illness may change our daily routine, did your routine get affected? How do TB patients set their routine and what was the role of family members in day-to-day activities? c) Now that you have this illness for the last few months, how do your family members cope with your disease and treat you at home?'

These questions helped us in gaining information about patients' daily routine, role of family, their attitude and level of support and care provided by family. When question was thrown out for discussion, everybody tried to give a positive impression of their family. According to the participants, families were very co-operative and sensitive to their needs. Majority of them were reluctant to express negative feelings about their families. Probing and appeal to share positive as well as negative experiences brought out the real information.

Patient's expectations about family support and care

Participants unanimously mentioned that women in the household should take care of TB patients. Most of the female patients reported discrimination and alienation in day-to-day life and mentioned 'nobody' to take care. It was expressed that married female patients face non-cooperation from in-laws and also received unsympathetic treatment. Most of the respondents mentioned that unmarried female patients have parents or siblings to remind about medicines, serve food in the bed and, if necessary, to take them to hospital. Married women could not expect all this; neither husband nor other members were willing to help. A female patient aged 46 years expressed that, '…. men do not have patience to take care of a sick person, by nature they are rowdy. They cannot be as warm as women. My husband accompanies me to the hospital. However, he would not care to ask if I have eaten meal. I expect him to have understanding about my illness and suffering, show sympathy and speak at least a few good words that will reduce my stress. But my expectations are futile.'

According to one of the female participants aged 24 years who was feeling very awkward to comment but finally mentioned that , '…..being a female, family members expect you to do domestic as well as the farm work. For them 'getting the work done' is more important than individual. I receive no help in my daily work, except in cooking. Mother-in-law has taken over cooking after learning about my illness. I take care of other domestic work like washing and cleaning and other household activities. I cannot ask for help, though I want them to give me some relief from work. Days when I am very sick, I do not get up at all. Other days, I do my work slowly and sleep under a shade on the farm.'

A middle-aged female, who was deserted by her husband and was feeling depressed, was very articulated and narrated a few incidences which forced her to leave husband's house. ' ….daily routine is not affected much but interaction with other members in family, kin group and neighbours definitely undergo changes. Everything cannot be explained in words. One has to feel and go through the experience. It is difficult to express isolation in words but food is served last, touch is avoided, even words are used sparingly, we can read rejection in their eyes. Overnight change in attitude and behaviour of family members can be observed'. She demanded alternative staying

arrangements for affected women who might get deserted and need somebody to rely on. She continued, '…. it is not possible to go and stay with parents, always. There is a need to have somebody to pat on our back and encourage to lead life with the same vitality.'

Sympathetic environment that is referred to as 'care' and physical help that is 'support' was more readily available for male patients as compared to female patients. This impression was created during FGDs of females. During FGDs of male patients, following information was gathered, majority said that, '….women cannot just sit and rest whole day because they have responsibility of the entire household. They have to cook and feed children and husband. They are required to do washing and cleaning. Therefore, care received by men and women would always remain different. However that does not mean that men are not discriminated.'

As narrated by a male TB patient aged 57 years, who was a farmer and had irrigated land, ' I have to demand for care and support from my family members. It did not come willingly to me. All three daughters in law refused to wash my clothes, give me food on time but I have learnt to get my work done from them.' Family members had to comply with his demands because the patient was the main bread earner of the family. He might be an exception and all other patients might not be able to command such a respect. Yet, rest of them listened carefully and nodded vigorously to some of his statements. Therefore, views expressed by other male members were very important to us in the analysis. In cases where the patient could not earn their daily wages due to the disease, their family members showed very less sensitivity towards their illness. Both males and females suffer same degree of stigmatization, discrimination and pressure from family. Following excerpt confirms this view, ' …we understand patient should complete treatment, but men constantly feel the mounting pressure of economic responsibility. We need food and meals for two times in a day. Who is going to bring it? They get caught between demands of families and their own health needs. Therefore they need motivation and assurance that recovery will happen soon.' This was narrated by a male patient, 48 years' old and was unemployed due to the illness. Some of the participants looked very disheartened and discussed these matters in a very depressing voice. One of the male participants aged 33 years felt, ' I have no idea what is going to happen after completion of the treatment. My wife has stopped respecting me and does not interact openly ever since she learnt about diagnosis. Fights over trivial matters have now become inevitable.' Participant aged 53, who owned a small piece of land believed, 'I got TB because of dust and working in sunlight for long hours every day. It is not my fault'. Participants arrived at conclusion that all TB patients received support and care but not necessarily 'best support and care'. Family's cooperation depended on the affected person's status and role in the family. There were very few young men who participated in FGD. Young men from poorer families with financial responsibility linked care and support to their deteriorating economic status. Financially dependent patients faced humiliation and embarrassment. Those who had regular income, demanded care and support from family members. These views were shared with lot of guilt and shame in their eyes. Regarding the acceptability of patients among extended family and relatives, most of them reported discrimination and alienation. A story of TB affected woman was narrated by one of the members. Young lady with two kids, was deserted by husband and she was sent back to her parents' house for the treatment. She recently came to know that her husband got remarried and now not willing to accept her. In such circumstances, very few relatives cared to visit or offer help in solving such problems. Those who came down showed lip sympathy and nothing beyond that. Most of the participants in that group profoundly agreed to the descriptions of behaviour of the relatives.

DISCUSSION

The major contribution of this particular research is clarity of concepts 'support and care' as described by TB affected individuals. We believed that these fifteen FGDs yielded the factual results in less time. This method has been proved as an useful

tool to collect information on the perceptions, beliefs, values and understanding of health issues and has been used by some of the studies in India [14] and outside.[15] It appears that the major barrier in assuming role of family is their unpreparedness, notions about TB, impact of stigma and discrimination. Earlier studies[16,17] have proved that in chronic conditions, family caregivers often feel unprepared to provide care, have inadequate knowledge to deliver proper care, and receive little guidance from the formal health care providers. Encouraging informed and active involvement of family in care, as seen in other diseases like cancer[18] may raise awareness and reduce stigma. It is a well-known fact that stigma attached with disease always hampers care and support. As evident from the research on HIV/AIDS,[19] stigma can lead to discrimination, denial of healthcare, employment, education, and other fundamental rights. In case of TB, family and community are guided by several such misconceptions[20] leading to stigmatization of the disease and hence, need to adopt support strategies that are required to enhance acceptance of patients.[21] As seen in the present study, instead of empathy and cheering pat on back, they experienced discrimination, neglect and disgrace. The findings are similar to that of a study done among women TB patients sometime back in the neighbouring geographical area.[22] The study has demonstrated that difference exists in the 'expectations' of patients and the 'real care and support' provided by family. This is how lack of support, stigma and discrimination contribute to the existing burden of TB at individual level. Research on families' involvement in TB programme is limited but there are some examples where families have shared responsibilities with public health system.[23] Research carried out in Tamilnadu[24] suggests that there is a need to alleviate fears from patients' mind by providing counselling and health education to community. We would like to include families prior to communities.

RECOMMENDATIONS

Following recommendations are provided in the light of above discussion and based on the existing information, education and communication (IEC) component of the RNTCP. Current IEC component[25] focuses on awareness raising, advocacy, social mobilization and improving patient provider communication. Families are one of the target groups in the health communication strategy, but nothing has been elaborated on how family should get involved and help TB patients complete treatment. Efforts to change family's attitude and behaviour towards tuberculosis patients are not specified. Families should understand the diverse needs patients may have during the long treatment period. They should be thoroughly educated and prepared to provide support to help reach the goal of completion of treatment. **Therefore, it is recommended that health providers should expand their role of health education and counsel family members to get more actively involved in the care of patients. If RNTCP staff faces problems in awareness building or involving family in care, a trained counsellor or a visiting psychologist is called for help. It is recommended that s/he should counsel such family members using various communication strategies and negotiate family's role in patient care. These techniques and education material used by counsellors can subsequently be handed over to the staff. This will definitely help us achieve objectives of RNTCP.**

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Case Report

TUBERCULOSIS OF LARYNX: A CASE REPORT

Manas Ranjan Rout[1] and Prabir Ranjan Moharana[2]

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Summary: A case of tuberculosis of larynx in a 45-year-old male patient has been described here. Usually, the signs and symptoms of laryngeal tuberculosis resemble with malignant diseases of larynx. The diagnosis was made here by the microscopic examination of sputum smear for Acid Fast Bacilli, chest x-ray, direct laryngoscopy and biopsy from the laryngeal lesion. **[*Indian J Tuberc 2012; 59:* 231-234]**

***Key words*:** Laryngeal Tuberculosis, Extra-pulmonary Tuberculosis, Odynophagia, Laryngoscopy.

INTRODUCTION

Involvement of larynx in tuberculosis occurs as secondary to pulmonary tuberculosis. Primary involvement of larynx is rare**.** Exact mode of transmission from the lungs is not known. It is believed that contact with sputum containing tubercle bacilli plays an important role. The occurrence of tuberculosis of larynx has greatly decreased as a result of improvement in public health care and development of effective antitubercular chemotherapy. These patients usually present with the symptoms of cough, hoarseness of voice, pain in throat, dysphagia, haemoptysis which simulate malignancy and other granulomatous infections of larynx.

This report describes a 45-year-old male patient with laryngeal tuberculosis who presented to us with symptoms of hoarseness of voice, productive cough, mild pain in throat and odynophagia.

CASE REPORT

A 45-year-old male patient came to our Out Patients' Department with complaints of hoarseness of voice and mild pain in throat since one month and pain during swallowing since ten days. During clinical history-taking, he revealed that he had cough with expectoration since four months. During this period, he had low grade of fever associated with gradual deterioration in health.

There was no previous history of similar illness and tubercular infection in the family. Patient was not an alcoholic but he was a known-smoker for the last 20 years consuming around 10 cigarettes per day.

Since the last four months, he had taken several courses of antibiotics and analgesics without any relief of symptoms.

On physical examination, he was found to have thin body built. There was no pallor and lymphadenopathy. Findings of systemic examination were normal. On local examination, the oral cavity and posterior pharyngeal wall were found to be normal. On indirect laryngoscopy, the epiglottis was so much congested and edematous (Fig.) that other parts of the larynx could not be visualized. On video laryngoscopy, the epiglottis, arytenoids, inter arytenoid region and ventricular bands were found to be congested and edematous. Small multiple ulcers were found over the arytenoids, inter-arytenoid region and epiglottis with purulent exudation. True vocal cord was poorly visualized. Movement of the vocal cords and arytenoids appeared to be normal with glottic chink due to edema of the arytenoids.

1. Assistant Professor (ENT, Head & Neck Surgery) 2. Assistant Professor (Community Medicine)
Alluri Sitarama Raju Academy of Medical Sciences, Eluru, West Godavari District (Andhra Pradesh)
***Correspondence*:** Dr Prabir Ranjan Moharana, Assistant Professor (Community Medicine), Alluri Sitarama Raju Academy of Medical Sciences, Eluru, West Godavari District (Andhra Pradesh) - 534 005; E-mail: drprabir2007@rediffmail.com; Mob-8985221941.

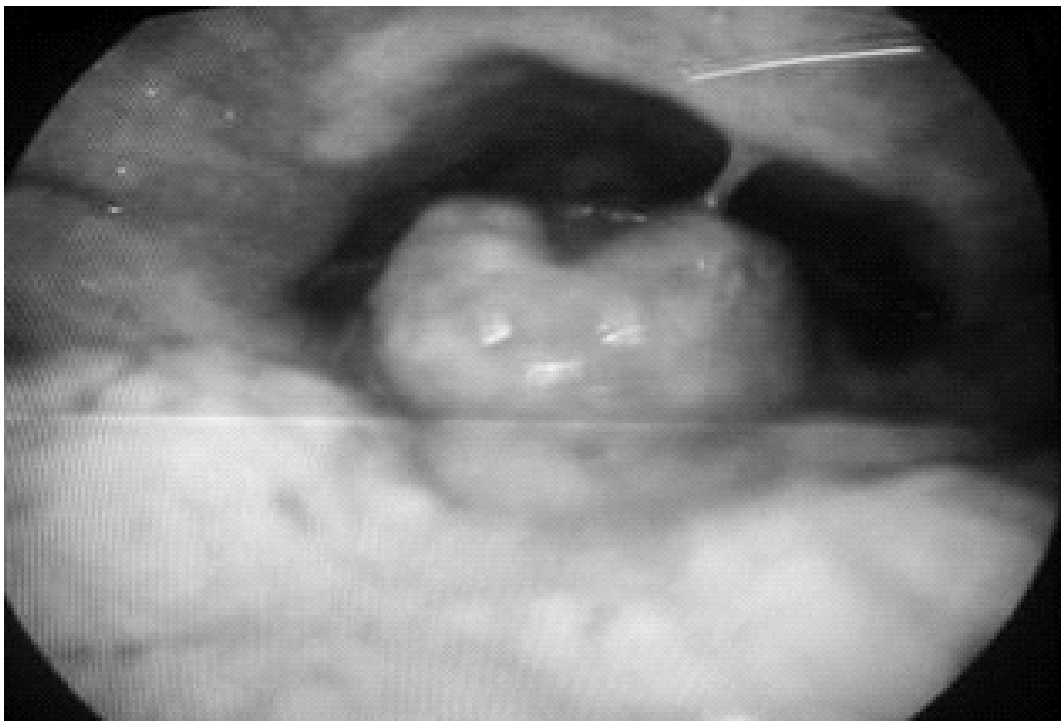

Figure: Congested and edematous epiglottis with multiple ulcers.

On examination, the nose, ear, head and neck were found to be normal. All the cranial nerves were functionally intact.

His laboratory investigations revealed normal haemoglobin level(13gm%), normal differential count (Neutrophils-65%, Lymphocytes-30%, Eosinophils-5%, Basophills-0%, Monocytes-0%), normal total leucocytes count (8500 cells/mm^3) and a raised Erythrocyte Sedimentation Rate (50 mm in first hour). Mantoux test revealed an induration of 12 mm after 72 hours. A chest radiograph showed patchy opacities in both the lung apices. Sputum smear was found positive for Acid Fast Bacilli. Tests for Human Immunodeficiency Virus(HIV) status and Hepatitis-B Virus(HBV) Surface Antigen were found to be negative. Liver function tests, renal function tests and fasting blood sugar were found to be within normal limits.

Direct laryngoscopy was done under short general anaesthesia and biopsy was taken from epiglottis and inter arytenoid region. The specimen was sent for histopathological examination.

The histopathological examination revealed fibro-collagenous tissue lined by stratified squamous epithelium enclosing fair number of confluent epithelioid cell granulomas with Langhan's type giant cells surrounded by lymphocytes and fibroblasts with a few areas of caseous necrosis suggestive of tuberculosis.

On the basis of bacteriological, radiological and histopathological findings, diagnosis of laryngeal tuberculosis secondary to pulmonary tuberculosis was established.

Then standard Category-I regimen of Revised National Tuberculosis Control Program (RNTCP) was given to the patient for six months. Follow-up examination after one month of treatment showed resolution of the signs and symptoms. Larynx appeared

normal under video laryngoscopy and there was significant gain in weight of the patient after two months of treatment.

DISCUSSION

During the last 10 years, mortality from tuberculosis has decreased by 43% in India.[1] Now the disease is changing its manifestation with increase in the incidence of extra-pulmonary cases. However, the cause of this change is not clearly known.[2] On the other hand, it might be due to an increased number of cases of extra-pulmonary tuberculosis which are being diagnosed by newer techniques.

Laryngeal tuberculosis is a rare clinical entity and recent incidence of laryngeal tuberculosis is less than 1% of all tuberculosis cases.[3] In a series of 843 tuberculosis cases, only 11 cases showed laryngeal involvement (1.3%).[4] But India is an endemic zone for tuberculosis. In a study of 500 patients with pulmonary tuberculosis from India, laryngeal involvement was observed in 4% of them.[5]

Laryngeal tuberculosis may be primary or secondary to pulmonary tuberculosis. Primary laryngeal tuberculosis occurs without any evidence of pathology in lungs or in any other site. Present case was thought to be secondary to pulmonary tuberculosis.

Male predominance is found in laryngeal tuberculosis i.e. 2-3:1 and the commonest age group is 40-60 years.[6]

Tuberculosis in head and neck region is commonly associated with HIV infection. In any HIV positive patient with head and neck lesion, tubercular infection is to be excluded first.[7] Now the incidence of tuberculosis is increasing because of co-existing HIV infection. In this case, the patient was negative for HIV infection.

Alonso *et al,* in their report of 11 laryngeal tuberculosis cases, found 'isolated dysphonia' or 'dysphonia with odynophagia' to be the most common presenting symptom(s).[8] In our case, the presenting symptoms were hoarseness of voice, pain in the throat, odynophagia and productive cough.

In laryngeal tuberculosis, anterior part of larynx is more commonly involved than posterior part and the most common site of involvement is inter arytenoid region.[9] But according to Clery and Batsakis, involvement of anterior half of larynx now occurs twice as often as the posterior half of larynx. The vocal cords are the most commonly affected sites(50-70%) which are followed by false cords(40-50%), epiglottis, aryepiglottic folds, arytenoid, posterior commissure and sub-glottis (10-15%).[10] In this case, the involved sites were epiglottis, arytenoids, inter arytenoid fold and ventricular bands.

The findings of laryngeal tuberculosis can be categorized into four groups i.e. (a) whitish ulcerative lesions (40.9%) (b) non-specific inflammatory lesions (27.3%), (c) polypoid lesions (22.7%) and (d) ulcero fungative mass lesions (9.1%).[11] The present case showed whitish ulcerative lesion over the arytenoids, inter arytenoid region and epiglottis with purulent exudations.

Direct laryngoscopy and biopsy are mandatory to establish the confirmative diagnosis. It can be done under local or general anaesthesia. Characteristic features which are found in tuberculosis are epitheloid granulomas with Langhan's type of giant cells and caseating granuloma formation.

It should be kept in mind that tuberculosis and malignancy of larynx may co-exist.[12] So, biopsy not only diagnoses tuberculosis, but also excludes malignancy as early as possible. Anti-tubercular therapy offers a good prognosis. This patient became asymptomatic after one month of chemotherapy.

CONCLUSION

Laryngeal tuberculosis is no more a rare condition with incidence of 4% among all cases of tuberculosis. In most of the cases, it is secondary to pulmonary tuberculosis. Direct laryngoscopy and biopsy are mandatory to establish confirmatory diagnosis and to exclude malignant diseases which often co-exist. Anti-tubercular therapy is the treatment of choice and prognosis is very good if it is treated early.

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Case Report

MULTIPLE TUBERCULOUS ABSCESSES AND MEDIASTINAL LYMPHADENITIS WITH NO PULMONARY INVOLVEMENT IN AN IMMUNOCOMPETENT PATIENT

K. Samitas[1], E. Marinakis[2], C. Birbilis[2], L. Thanos[3], E. Zervas[1], M. Gaga[1] and E. Diamanti-Kandarakis[2]

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Summary: Tubercular cold abscesses secondary to neighbouring bone involvement are a well-known clinical manifestation of extra-pulmonary tuberculosis. However, primary soft tissue tuberculous abscesses with no pulmonary involvement in immuno-competent patients are very uncommon. A rare case of multiple primary intrathoracic and extraperitoneal soft tissue tuberculous abscesses and mediastinal lymph node tuberculosis with no pulmonary involvement is reported. This case demonstrates the need for a high index of suspicion for such rare presentations of extra-pulmonary tuberculosis in patients from endemic areas. **[*Indian J Tuberc 2012; 59:* 235-239]**

INTRODUCTION

Tubercular cold abscesses secondary to neighbouring bone involvement are a well known clinical manifestation of extrapulmonary tuberculosis (EPTB). However, primary skeletal muscle abscesses with no bone involvement in immuno-competent patients are very rare[1,2]. To our knowledge, multiple intrathoracic and extraperitoneal soft tissue tuberculous abscesses and mediastinal lymph node tuberculosis (LNTB) with no pulmonary involvement in immunocompetent patients have not been previously reported.

CASE REPORT

We present a case of a 19-year-old Somali male who had illegally immigrated to Greece three months prior to admission. He had suffered of prolonged fever up to 39^{o}C for at least two months and weight loss (eight kg in three months) and complained of night sweats and lower back pain. He was a non-smoker and had no previous hospitalizations. Physical examination was without pathological findings, apart from pain in the right flank and gluteal regions, with no palpable masses. The blood work revealed mild normochromic anaemia (HCT: 34.5%, HGB: 11.0 g/dl) and increased inflammatory markers (ESR: 120 mmHg/h, CRP: 10.4 mg/dl). White blood count and CD4 cells were normal. Serology for HIV and hepatitis was negative. Chest X-rays were unremarkable (Figure 1A), while an abdominal ultrasound did not demonstrate any pathology. An empirical treatment with antibiotics was initiated (clindamycin 600mg thrice daily IV). Blood cultures for aerobic and anaerobic bacteria did not produce any growth. Mantoux test was positive (15mm). After five days of treatment, the patient didn't show any improvement and was subjected to further examinations.

A computed tomography (CT) of the abdomen revealed abscesses in the vicinity of the right psoas and iliacus (Figure 2A), internal obturator (Figure 2B), middle gluteal (Figure 2C) and internal oblique muscles (Figure 2D), without any lymph node involvement. In the osseous window, minor lytic lesions were observed in the outer edge of the right iliac crest, near the iliacus and gluteal abscesses (Figure 2E and 2F). No bone lesions were found in the bony structures near any other observed cold abscesses. No other pathological findings were observed in the upper or lower abdomen. A CT of the thorax was also performed, which demonstrated an

1. 7th Respiratory Department and Asthma Centre 2. 3rd University Internal Medicine Department 3. Radiology Department
Athens Chest Hospital, Athens, Greece
***Correspondence*:** Dr. Konstantinos Samitas, MD, PhD, 7th Respiratory Dept and Asthma Centre, Athens Chest Hospital, Mesogeion 152, 11527, Athens, Greece; Tel: +302107781720; Fax: +302107781911; Email: k.samitas@yahoo.com

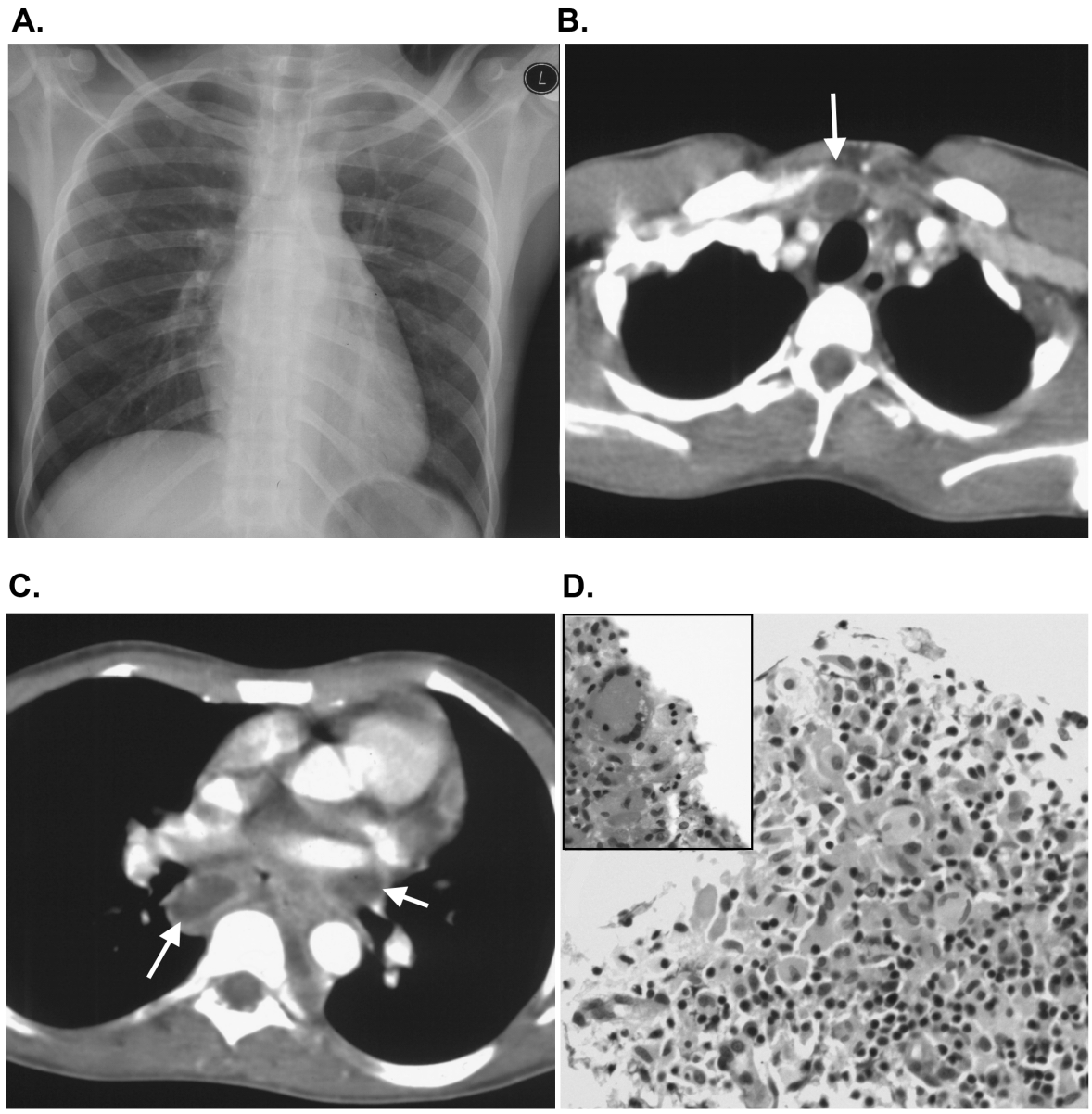

Figure 1: Chest X-ray with no pathological findings (A). CT scan of the thorax showing a mediastinal abscess (low-density area with peripheral enhancement) originating in the subclavicular fossa and extending posterior until the upper third of the sternum (white arrow) (B), as well as enlarged subcarinal lymph nodes with cystic degeneration (white arrows) (C). In (D) a representative peripheral part of an epithelioid granuloma is depicted from the transbronchial needle biopsy specimen. Caseous necrosis is absent (H.E. x200). The inset on the upper left demonstrates in greater detail a multinucleated giant cell at the periphery of the granuloma (H.E. x400).

additional abscess originating in the subclavicular fossa and extending posterior until the upper third of the sternum (Figure 1B), with no bone lesions in neither the sternum nor ribs. Enlarged subcarinal lymph nodes with cystic degeneration were also observed (Figure 1C), with no pathologic findings in the lung parenchyma.

The patient was subjected to CT guided fine-needle aspiration (FNA) of the right gluteal and psoas abscesses. The aspirated fluid, a yellowish cheesy liquid, was sent for mycobacteriological analysis, including liquid (Bactec MGIT 960) and Löwenstein-Jensen cultures, as well as polymerase chain reaction (PCR) for *M. tuberculosis* (AMTD, GenProbe-BioMerieux). The patient was subsequently subjected to fiberoptic bronchoscopy with no intrabronchial findings. Washing and brushing as well as transbronchial needle biopsies (TBNB) from the pathological lymph nodes (LN7) were obtained.

Pus cultures did not produce any growth and Ziehl-Neelsen staining was negative for acid-fast bacilli. The histopathological TBNB examination showed early-forming epithelioid granulomas with elements of non-caseous necrosis, giant cells and

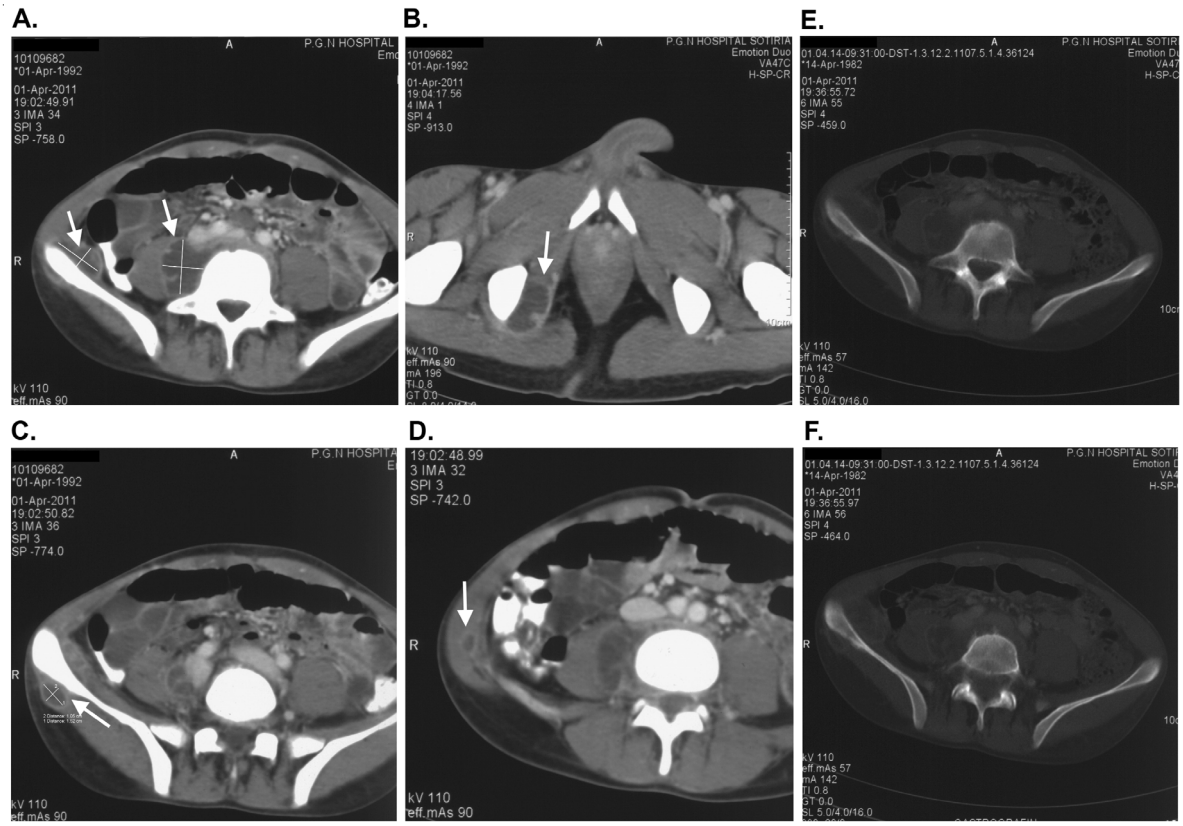

Figure 2: Computed tomography (CT) scan of the abdomen depicting (with white arrows) well defined abscesses (low density areas with peripheral enhancement after intravenous administration of contrast media) in the vicinity of the right psoas and iliacus (A), internal obturator (B), middle gluteal (C) and internal oblique muscles (D). Finally minor lytic lesions can be observed in the outer edge of the right iliac crest, near the iliacus and gluteal abscesses in (E) and (F).

lymphoepithelioid infiltrates (Figure 1D). While still waiting for PCR and cultures for *M. tuberculosis*, anti-tuberculous (anti-TB) therapy was initiated on the basis of high probability for EPTB. Due to the patient's origin and high probability of drug resistance to first-line agents[3,4], including streptomycin, a five-regimen treatment was initially selected (isoniazid 5 mg/kg, rifampicin 10 mg/kg, pyrazinamide 25 mg/kg, ethambutol 20 mg/kg and amikacin 15mg/kg).

MGIT cultures and PCR analysis came out negative but treatment was continued, as the patient showed partial improvement of symptoms (no fever, reduced pain). Since EPTB was not still certain, a low-dose CT scan of the abdomen was performed three weeks after anti-TB treatment to evaluate possible radiological deterioration due to other (i.e. microbial) causes. Partial reduction in the abscesses' dimensions was observed. After five weeks, the results of Löwenstein-Jensen cultures came out positive for *M. tuberculosis* (sensitive to all first-line drugs). Treatment with amikacin was stopped and the patient continued therapy with the remaining four drugs. The patient was discharged asymptomatic under supervision from the Tuberculosis Out-patient clinic for direct observation therapy (DOT).

The patient was followed up for six months after his discharge, during which he demonstrated gradual clinical improvement. During his last visit, the patient presented with no pain in the gluteal and flank regions and had gained weight (5kg). The follow-up of the abscesses was performed by means of ultrasound and were found to have gradually decreased in size. Unfortunately, the patient did not show up for the next scheduled follow-up visits and could not be reached in the address he had provided.

DISCUSSION

In developed countries, up to 20% of TB cases have extra-pulmonary involvement, but in patients from high-incidence countries, the rate is much higher[5]. Developed countries with low incidence of TB have experienced increased cases of recurrent TB during the last decade, primarily due to the increasing number of immigrants from developing countries[3,4]. Our patient had recently illegally emigrated from Somalia. Tuberculosis is considered to be a major public health problem in this country[6]. A high percentage of recurrent TB with high incidence of EPTB and high drug resistance, have been previously reported in Somali immigrants[3,4]. Due to the increased probability of drug resistance to first-line agents as well as streptomycin, a five-regimen treatment that included amikacin, usually efficient in MDR-TB, was selected. Upon confirmation of pansensitivity, amikacin was discontinued.

Isolated primary mediastinal tuberculous abscesses are rare[7,8] and musculoskeletal TB occurs in only 1%-3% of patients, most commonly affecting the spine. Extraspinal musculoskeletal TB is among the least common manifestations of TB[5]. Our patient presented with EPTB accompanied by mediastinal LNTB and multiple intrathoracic and abdominal cold abscesses. Minor lesions were observed in the outer edge of the right iliac crest, which may have given rise to neighboring tuberculous abscesses; however, no bone involvement was observed in the thoracic vertebrae, sternum and ribs near the mediastinal abscess.

The diagnosis of EPTB, especially involving deeply located, hard to access areas is very difficult. CT and/or gadolinium-enhanced MRI are very useful in the diagnosis of intramuscular abscesses; however tissue is needed to confirm the diagnosis. CT guided FNA is particularly helpful in providing biological material for establishing a diagnosis. If mediastinal lymph nodes are involved, TBNB is the least invasive technique that can provide additional tissue for diagnostic purposes. The clinical utility of PCR in the diagnosis of extra-pulmonary tuberculosis has been very well documented[9,10]. However, as in our case, it is often negative, especially in purulent biological material. Biopsy and cultures still remain the gold standard.

This case report presents a rare manifestation of extra-pulmonary TB with mediastinal lymphadenitis accompanied by multiple mediastinal and extraperitoneal tuberculous soft tissue abscesses with no pulmonary involvement. Clinicians should remain continuously aware for early detection and

treatment of EPTB in patients from endemic areas, since unusual or uncommon presentations of this treatable infection could delay diagnosis and treatment with adverse sequel for the individual and the community.

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Case Report

ORO-FACIAL GRANULOMATOSIS - A CASE REPORT

Rohit Jaiswal[1], Sarita Chaudhary [2], Priyanka Singh[2] and Manvi Gupta[3]

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Summary: Oro-facial granulomatosis includes a group of disorders which present as a chronic, non-ceasating granulomatous lesion involving the perioral tissues of face and oral mucosa. This condition was first referred by Mart in 1859, Hubschmann in 1894 and Luscher in 1949; later, Wiesenfeld in 1985 introduced the term "Oro-facial Granulomatosis" (OFG) which is characterized by persistent or recurrent soft tissue enlargement and oral ulceration. Hence, it is important to establish the diagnosis accurately because this condition sometimes is a manifestation of Crohn's disease or Sarcoidosis. A case of Oro-facial granulomatosis in a 15-year-old male is reported. **[*Indian J Tuberc 2012; 59:* 240-242]**

Key words: Lip, Multinucleated Giant cells, Granuloma

INTRODUCTION

OFG is an uncommon immunologically mediated disorder clinically characterized by recurrent or persistent swelling of the oro-facial tissues and oral mucosal ulceration.[1] The chronic inflammation of OFG is often characterized by the presence of non-caseating granuloma in the sub-epithelial stroma.[2,3] Although several areas of the face and oral cavity can be affected,[4,5] the classic and most frequent clinical feature of OFG is a painless, non-erythematous, non-pruritic edema of the lips.[6] After recurrent attacks at irregular intervals, the swelling becomes firm and indurated.[7]

CASE REPORT

A 15-year-old male reported to the Department of Oral Medicine with the chief complaint of swelling on his upper and lower lips since five years {Fig.1}. The patient's past medical history was non-contributory. There was no evidence of foreign material or trauma and patient had undergone prolonged histamine and antibiotic therapy before referral. On examination, a well-defined swelling involving the upper and lower lips was present measuring up to 5 x 2 cm in size with overlying skin being stretched, shiny and pinkish in colour. On palpation, swelling was non-tender and firm in consistency. Radiographic studies of the face and the chest were followed by hematological investigations which included erythrocyte sedimentation rate, hemoglobin estimation, total leucocyte count, later serum Calcium and Angiotensin Converting Enzyme tests were done which were found to be within normal limits, other investigations

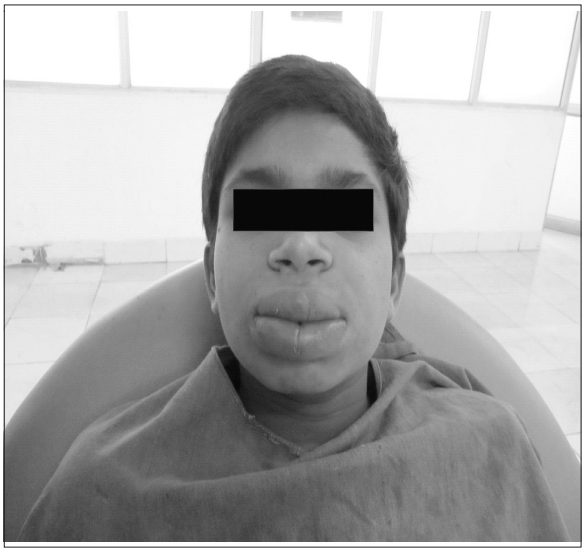

Fig. 1: Swelling of the upper and lower lip.

1. Reader 2. Lecturer 3. P G student
Department of Oral Maxillofacial Pathology, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow (Uttar Pradesh)
***Correspondence*:** Dr. Rohit Jaiswal, F1/B1 River Bank Colony, Lucknow-226018 (Uttar Pradesh); Mobile: 09935108813; Phone: 0522 - 2615630; E-mail: rohitjaiswal75@rediffmail.com; Fax no.: 0522 - 2440335

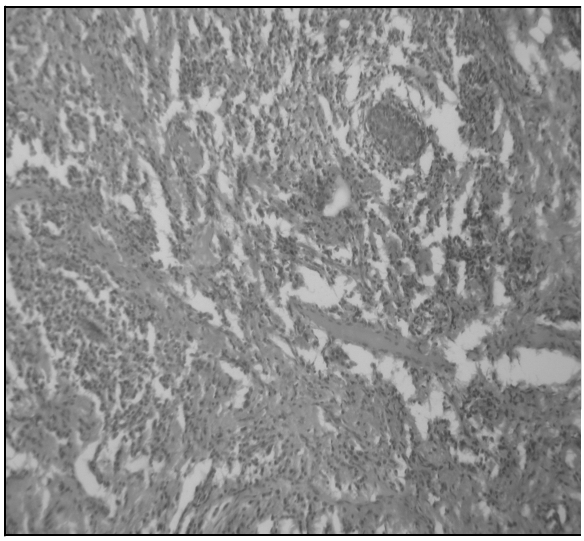

Fig. 2: Multi-nucleated giant cells with perivascular rimming of lymphocytes (10X) (*arrows*) (hematoxylin and eosin [H&E]; magnification lower lip.

like Mantoux test were ruled out as the patient did not show any clinical signs of Hemoptysis, allergy or drug reaction. Hence, a provisional diagnosis of angioedema or Oro-facial granulomatosis was made. The patient was later advised for an incisional biopsy. Microscopic examination revealed the presence of non-ceasating granuloma composed of epithelioid and giant cells with a peri-vascular rim of lymphocytes [Fig. 2]. Hence, a histopathological diagnosis of OFG was made based on the findings. Later, the patient was administered prednisolone 1mg/kg for four weeks followed by a slow tapering over two to three months to suppress the lesion. The patient is symptom-free since then and is being followed up periodically for possible recurrence.

DISCUSSION

OFG comprises a group of diseases characterized by non-caseating granulomatous inflammation affecting the soft tissues of the oral and maxillofacial region.[8] The precise cause of OFG is unknown.[9] Several theories have been suggested, including infection, genetic pre-disposition and allergy while other researchers have identified a monoclonal lymphocytic expansion in OFG lesions and have suggested that it could be secondary to chronic antigenic stimulation.[10] It appears that cytokine production by the lymphocytic clone could be responsible for the formation of granulomas in these lesions.[11] The classic presentation of OFG is a non-tender recurrent labial swelling that eventually becomes persistent.[6] This swelling may affect one or both lips, causing lip hypertrophy (macrocheilia)[12] which is very similar to our findings. The swelling is initially soft but becomes firmer with time as fibrosis ensues. Patients with oral complaints could suffer from linear and aphthous ulcers, or hypertrophy of the oral mucosa. A "cobblestone" appearance of the oral mucosa is a common presentation. Histologically, OFG is characterized by non-caseating epithelioid granulomas with or without multinucleated giant cells along with diffuse and focal aggregates of small lymphocytes (Wiesenfeld *et al*, 1985). Management of OFG can prove very difficult. Recent interest has focused on the role of elimination diets, particularly cinnamon and benzoate avoidance, with reports of improvement in as many as two-thirds.[13] Topical therapy (e.g. tacrolimus) and intra-lesional steroids are used in some cases.[14] Systemic immunosuppression, usually with a thiopurine, has been used for refractory OFG.[15]

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Short Communication

KNOWLEDGE AND ATTITUDE TOWARDS TUBERCULOSIS AMONGST THE TRIBAL POPULATION OF JHABUA, MADHYA PRADESH

V.G. Rao, R. Yadav, J. Bhat, B. K. Tiwari and M. K. Bhondeley

(Received on 2.5.2012; Accepted after revision on 13.8.2012)

Summary: A community-based cross-sectional survey was carried out amongst the tribal population of Jhabua in Madhya Pradesh. A total of 1385 individuals from randomly selected villages were interviewed using structured questionnaire. Eighty five per cent respondents had knowledge of the symptoms of tuberculosis. About a quarter of the respondents were not aware of any method for preventing TB. Though majority of the respondents (68.2%) stated that TB is a curable disease, most of them (67.2%) were not aware of the DOTS programme. The study emphasizes the need for extensive health education programme to create awareness about tuberculosis in tribal population of the region. **[*Indian J Tuberc 2012; 59:* 243-248]**

Key words: Tuberculosis, Tribal, Jhabua, Madhya Pradesh

INTRODUCTION

Tuberculosis (TB) is one of the most serious public health problems in the country. India accounts for nearly 30% of all tuberculosis cases in the world today and more adults in India die of TB than from any other infectious disease.[1] In 1998, the Indian government started large-scale implementation of Revised National Tuberculosis Control Programme (RNTCP) using the directly observed treatment, short-course (DOTS) strategy recommended by the World Health Organization.[2] Early passive case-finding and treatment compliance are the cornerstones of TB control programme. Despite this, the disease continues to be a serious public health challenge. It remains predominantly a disease of the disadvantaged and marginalized.

Tribal communities are underprivileged group of the society. The studies carried out among some tribal groups in the country show moderate to high prevalence of TB among them.[3-8] Strengthening of the tuberculosis services is required to care for these socially isolated and hard-to-reach groups. However, success of TB control activities depends on the correct knowledge and positive perception of the community towards TB and its management. Another serious problem is stigma associated with TB leading to delayed treatment seeking and poor adherence to therapy.

The present study was carried out among tribal population of Jhabua district - mostly the Bhils and Bhilalas. Jhabua is predominantly a tribal district located in the western part of Madhya Pradesh. About 85% of the population is tribal. The main aim of this study was to describe the knowledge of TB among tribal men and women and to see their health seeking behaviour.

MATERIAL AND METHODS

This cross-sectional study was carried out in seven tribal villages of two randomly selected taluqs of Jhabua district (of the total 302 tribal dominated villages) during Aug- Sept 2007. The study population consisted of tribal men and women- mostly the Bhils and Bhilalas. A sample size of 1363 was estimated considering an expected proportion (knowledge about TB) of 50%, margin of error 1.5% at 95% confidence level. A village was considered as a sampling unit for the survey and the required number of villages was selected randomly to cover the required sample size.

All men and women above the age of 20 years were included in the study. A structured questionnaire,

Regional Medical Research Centre for Tribals (Indian Council of Medical Research), Jabalpur (Madhya Pradesh)
Correspondence: Dr. V. G. Rao, Scientist F, Regional Medical Research Centre for Tribals (Indian Council of Medical Research), Nagpur Road, P.O. Garha, Jabalpur - 482 003 (Madhya Pradesh); Ph: (O)+ 91 761 2370800, 2672447; Fax: + 91 761 2672835; Email: drvgrao@rediffmail.com

which consisted of the basic socio-demographic characteristics and the questions, related to the knowledge and attitude of the study population towards tuberculosis was prepared in the local language (Hindi) and was tested in the field area. Interview technique was used to collect the information from the study participants. Prior to data collection, a good rapport was established with the community leaders including local officials by explaining the aims and objectives of the study. Informed consent was obtained from the individuals participating in the survey.

RESULTS

A total of 1385 tribal men and women were interviewed. The socio-demographic characteristics of these subjects are given in Table 1. Majority of the study subjects (60.3%) were in the age-group of 20-

Table 1: Socio-demographic characteristics of study population

Characteristics	Frequency (n=1385)	Per cent
Age group		
20-40	835	60.3
41-60	359	25.9
≥ 61	191	13.8
Sex		
Male	859	62
Female	526	38
Size of households		
1-2	58	4.2
3-4	277	20
5-6	410	29.6
7-8	409	29.5
≥ 9	231	16.7
Occupation		
Agriculture labour	1086	78.4
Other Labour	274	19.8
Service	25	1.8
Yearly family income (INR)		
≤ 10000	897	64.8
10001-20000	432	31.2
>20000	56	4.0
Education		
Illiterate	1116	80.6
Primary	135	9.8
Middle	65	4.7
High school & above	69	4.9

40 years. Most of them (>78%) were agricultural labourers and majority (65%) had annual family income of less than Rs. 10000. More than 80% subjects were illiterate, while about 10% had received primary education.

Table 2 shows the knowledge of the study subjects regarding symptoms, causes and prevention of tuberculosis. Majority of the (85.4%) respondents were aware of the signs and symptoms of the disease. The commonest symptoms mentioned by the study subjects were cough (59.0%), chest pain (56.8%) and haemoptysis (50.1%). Less frequent responses included fever (25.2%), weakness (17.6%) and weight loss (11.2%). Most mentioned smoking (49.6%) and alcohol consumption (46.5%) as the causes of TB. About a quarter of the respondents know that the disease is caused by the germs. Some of the other responses as the causes of TB were polluted water, unhygienic conditions, poverty, etc. Some respondents also mentioned heredity as the cause for TB. The most common idea to prevent TB was to avoid smoking (30.3%). One hundred and eighty one (20.9%) respondents stated that TB could be prevented

Table 2: Knowledge regarding the symptoms, causes and prevention of TB

Knowledge	Number of Respondents (n=867)	Per cent
Symptom		
Cough	511	59.0
Chest Pain	493	56.8
Haemoptysis	435	50.1
Fever	219	25.2
Weakness	153	17.6
Weight loss	97	11.2
Don't Know	127	14.6
Cause		
Germs of TB	207	23.9
Polluted water	230	26.5
Smoking	430	49.6
Alcohol consumption	403	46.5
Living in unhygienic conditions	124	14.3
Others	86	9.9
Don't Know	114	13.1
Prevention		
Avoid smoking	263	30.3
Avoid people who have TB	181	20.9
Avoid alcohol	145	16.7
Avoid hard work	84	9.7
Others	54	6.2
Don't Know	202	23.3

Table 3: Knowledge and attitude related to TB treatment

Characteristics	No. of Respondents (n=867)	Per cent
Treatment		
Use modern medicine	465	53.6
Use traditional medicine	211	24.3
Nutritious food	142	16.4
Others	79	9.1
Know that cure of TB is possible		
Yes	591	68.2
No	86	9.9
Don't Know	190	21.9
Know about DOTS		
Yes	284	32.8
No	583	67.2
Preferred choice of treatment		
Government facility	622	71.7
Private hospitals/ clinics	189	21.8
Others	56	6.5

by avoiding people who had the disease. Less common beliefs were to avoid alcohol, hard work, sharing objects used by a TB patient and to eat nutritious foods. BCG as a method of TB prevention was mentioned by twenty two (2.5%) individuals. Many (23.3%) were not aware of any method for preventing TB. The knowledge of the respondents regarding treatment showed that the majority (53.6%) were receptive to use of modern medicine (Table 3). Reference to traditional medicines was made by a few respondents (24.3%). Other choices included nutritious food (16.4%), praying (3.7%) and rest (1.2%), etc. Though majority of the respondents (68.2%) stated that TB is a curable disease, most of them (67.2%) were not aware of the DOTS programme. Majority preferred government facility (71.7%) for treatment of tuberculosis.

DISCUSSION

The tuberculosis services mainly focus on bacteriological cure of the disease. Understanding patient's beliefs about TB would be useful for better programme performance. The present study reveals that the tribal community of Jhabua District, by and large, posseses limited knowledge about various aspects of tuberculosis. Awareness about the signs and symptoms of a disease prompts patients to seek early treatment. The responses regarding the symptoms of TB in this community indicate a fairly good knowledge. A large number of Bhils knew cough, chest pain and haemoptysis as the main symptoms of tuberculosis. The symptoms enumerated by the respondents are fairly consistent with the symptoms of pulmonary TB. In Andhra tribal

study, Uplekar *et al* reported cough, haemptysis and fever as symptoms of tuberculosis by 66%, 13% and 6% respectively.[9] The awareness of TB symptoms in this community may be useful in passive case finding.

Lack of knowledge about the cause and prevention of tuberculosis is a matter of concern. Though only 23.9 per cent respondents knew that tuberculosis is caused by germs of TB, equal proportion of respondents were also of the view that tuberculosis is caused by polluted water (26.5%). Majority of the respondents attributed tuberculosis to smoking (49.6%) and alcohol consumption (46.5%). In Andhra tribal area study, 38% cases attributed the disease to tubercle bacilli.[10] The preventive measures enumerated by the respondents were avoiding smoking (30.3%), alcohol (16.7%) and people with TB (20.9%). About a quarter of the respondents were not aware of any preventive measure.

In this study, it was noted that despite the socio-economic background of the Bhils, their attitude towards health and health facilities was in favour on modern medicine. Though a quarter of the respondents still believe in traditional medicines for TB treatment, majority (53.6%) accept the modern medicine as the proper treatment for TB. Nutritious food was also mentioned by 16.4% respondents. More than seventy per cent respondents preferred government facility for TB treatment. Majority (68.2%) knew that TB is curable. This is an encouraging sign. The availability of health facilities and accessibility to health personnel should be strengthened to maintain and further improve this encouraging positive sign. In a study on health seeking behaviour in Zimbabwe, Cavender stressed the broader spectrum of health care including traditional medicine.[11]

Directly Observed Treatment-Short-course (DOTS) is the globally recommended standard of care in treatment of tuberculosis. The Revised National Tuberculosis Control Programme (RNTCP) is based on the principles of DOTS which is central to the success of RNTCP in the country. Awareness and the utilization of DOTS by the community therefore assumes significance. Majority of the respondents in the present study (67.2%) were not aware of the DOTS programme. This lack of awareness about the programme may have immense impact on the passive case detection, failure and relapse. There is an urgent need to create awareness among Bhils and Bhilalas about the availability and usefulness of the quality DOTS programme.

The limitations of the study need to be considered while interpreting the results. As the study was done in a small sample of the wider tribal population in the state of Madhya Pradesh, the results are not generalizable to the tribal population in the state. The study however, reveals the knowledge and attitude of tribal population of Jhabua district - mostly the Bhils and Bhilalas about various aspects of tuberculosis.

To conclude, the results of the present study highlight many lacunae in knowledge and attitude towards tuberculosis amongst Bhils of Jhabua district. The study emphasizes the need for extensive health education programme to create awareness and remove myths about tuberculosis in tribal population of the region.

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ABSTRACTS

Absolute lymphocyte count as a surrogate marker for CD4 counts after six months of HAART initiation in a resource-limited setting in India

S Srirangaraj and D Venkatesha. *Indian Journal of Medical Research* 2012; **135(6)**: 895-900.

Owing to the ever-expanding access to HAART (highly active anti-retroviral therapy) in resource-limited settings, there is a need to evaluate alternate markers like absolute lymphocyte count (ALC) as a surrogate for CD4 counts. This study was done to assess the usefulness of ALC as a surrogate marker for CD4 counts in monitoring HIV-infected patients after HAART initiation. In this study, 108 HIV-positive adult patients of both sexes fulfilling the inclusion criteria were included. CD4 and ALC were recorded at baseline. After initiation on HAART, these patients were followed up at three month intervals. ALC and CD4 counts were positively correlated (Spearman correlation coefficient= 0.553). After six months of HAART, the sensitivity of an ALC increase as a marker for CD4 count increase at six months was 82 per cent, specificity was 100 per cent, PPV was 100 per cent and NPV was 31 per cent. Area under the corresponding ROC curve for CD4 increase of >100 cells/î l was 0. 825 ± 0.053. ALC may be a useful surrogate marker in predicting an increase in CD4 counts as a response to HAART, but of questionable value in predicting a decrease in CD4 counts.

Yield of interview screening and chest X-ray abnormalities in a tuberculosis prevalence survey

N.B. Hoa, F.G.J. Cobelens, D.N. Sy, N.V. Nhung, M.W. Borgdorff and E.W. Tiemersma. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(6)**: 762-7.

Tuberculosis (TB) prevalence surveys generally rely on a combination of screening methods to identify suspects eligible for sputum culture. The objective was to assess the yield of screening methods applied in a recent prevalence survey in Viet Nam and estimate the proportion of TB cases missed due to incomplete participation. TB suspects were identified based on self-reported TB history or productive cough by interview and chest X-ray (CXR). We calculated the case yield of these two screening methods by dividing the number of cases detected per method by the total number of cases detected. As not all participants underwent the full screening procedure, we recalculated the maximum yield of the screening methods using multiple imputation methods. The yield from screening by interview and CXR were respectively 38% and 91%. Adjusting for missing data by multiple imputation, we estimated that we missed 9.9% (95% CI 6.8-14.2) of expected TB cases. In prevalence surveys, screening by pre-structured interview is insufficient, and should be supplemented with CXR to achieve sufficient identification of TB cases. The effect of incomplete participation in the full screening procedure may be substantial and should be adjusted for in the analysis.

Isoniazid for six months more cost-effective than isoniazid for nine months?

J.M. Pina, L. Clotet, M.R. Sala, A. Ferrer, C. Arias and A. Domínguez. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(6)**: 768-73.

The optimal treatment for latent tuberculosis infection consists of isoniazid (H, INH) for 9-12 months. Although INH for six months (6H) is more cost-effective than the 12-month regimen, the cost-effectiveness of the 6H regimen and that of INH for nine months (9H) have not been compared. The objective was to compare the cost-effectiveness of treatment with 6H and 9H. Cost-effectiveness was evaluated

using the ratio of the cost of preventing one tuberculosis case using 6H *vs*. 9H. The cost was estimated as the product of the number of patients to be treated to prevent one case using 6H or 9H× the cost of 6H or 9H. A total of 1039 patients were studied. The number of patients that needed to be treated to prevent one case was 33 (95% CI 21-83) using 6H and 26 (95% CI 18-50) using 9H. The cost of 6H and 9H was respectively 444.34 and 578.26, and the cost ratio of preventing one case with 6H/9H was 0.98 (95% CI 0.6-1.5). The cost-effectiveness of treatment with 6H and 9H is similar.

Alternative sputum preparation to improve polymerase chain reaction assay for *Mycobacterium tuberculosis* detection

C. L. Nogueira, L.M. Wildner, S.G. Senna, D. Rovaris, M.F. Gruner, A.R. Jakimiu, R.M. da Silva and M. L. Bazzo. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(6)**: 783-7.

Tuberculosis (TB), one of the major airborne infectious bacterial diseases, remains an important health problem worldwide. It is estimated that there are 1700 new cases per year in Santa Catarina State, Brazil. The objective was to improve polymerase chain reaction (PCR) sensitivity in detecting *Mycobacterium tuberculosis* in sputum samples. This study proposed the use of glass beads as a modification of the routine protocol for sputum preparation used in the Laboratory of Molecular Biology and Mycobacteria at the Federal University of Santa Catarina, Florianópolis, Brazil. The study comprised 120 sputum samples, 60 of which were treated with the routine protocol, while 60 were treated with the modified protocol using glass beads. Samples treated with the routine protocol had a sensitivity of 56.7% (95% CI 44.1-69.2) in 16S rRNA PCR and 81.7% (95% CI 71.9-91.5) in insertion sequence (IS) 6110 PCR, compared with culture. Samples treated with the modified protocol had a sensitivity of 73.3% (95% CI 62.1-84.5) in 16S rRNA PCR and 100% in IS6110 PCR. The modified protocol using glass beads greatly improved mycobacterial detection in sputum samples compared with the routine protocol.

Predictors of tuberculosis in first six months after initiation of antiretroviral therapy: a case-control study

R.N. Peck, A. Luhanga, S. Kalluvya, J. Todd, S. Lugoba, D. W. Fitzgerald and J. A. Downs. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(8)**: 1047-51.

In Africa, 10% of human immunodeficiency virus (HIV) infected adults starting antiretroviral therapy (ART) die within the first year, and tuberculosis (TB) is the leading cause of death. The objective was to investigate the predictors of ART-associated TB at an adult HIV clinic in Tanzania. In this nested case-control study, adults starting ART were screened for TB according to the World Health Organization protocol. Those not diagnosed with TB were observed for six months. Patients diagnosed with TB were defined as cases, and controls were selected from among the patients who did not develop TB using incidence density matching. Among the 2514 HIV-positive adults in our cohort, 72 (3%) were diagnosed with TB during the first six months of ART. By multivariate analysis, baseline characteristics predictive of TB were cough, fever and night sweats; 76% (55/72) of the cases had at least one of these symptoms at the time of starting ART. Overall, 75% of the patients who developed TB during the first six months of ART had TB symptoms at the time of starting ART. Improved TB diagnostics and/or better strategies for empirical anti-tuberculosis treatment are needed for patients with symptoms of TB at ART initiation.

Evaluation of an immunochromatographic test for discrimination between *Mycobacterium tuberculosis* complex and non-tuberculous mycobacteria in clinical isolates from extra-pulmonary tuberculosis

Anand Kumar Maurya**,** Vijaya Lakshmi Nag**,** Surya Kant**,** Ram Aawadh Singh Kushwaha**,** Manoj Kumar**,** Vikas Mishra**,** W Rahman and Tapan N Dhole. *Indian Journal of Medical Research* 2012; **135(6)**: 901-06.

Accurate diagnosis of tuberculosis (TB) is crucial to facilitate early treatment of the patients, and to reduce its spread. Clinical presentation of *Mycobacterium tuberculosis* complex (MTBC) and non-tuberculous mycobacteria (NTM) may or may not be the same, but the treatment regimen is always different for both the infections. Differentiation between MTBC and NTM by routine laboratory methods is time-consuming and cumbersome. This study was aimed to evaluate an immunochromatographic test (ICT), based on mouse monoclonal anti-MPT64, for simple and rapid discrimination between MTBC and NTM in clinical isolates from extra-pulmonary tuberculosis cases. A total of 800 clinical samples were collected from patients suspected to have extra-pulmonary tuberculosis. Preliminary diagnosis has been done by direct Ziehl-Neelsen (ZN) staining followed by culture in BACTEC system. A total of 150 clinical isolates, which were found positive in BD 460 TB system during September 2009 to September 2010 were selected for the screening by ICT test. p-nitro-á-acetylamino- â-hydroxy propiophenone (NAP) test was performed for differentiation of MTBC and NTM. *M. tuberculosis* complex was further confirmed by IS6110 PCR of BACTEC culture positive isolates, this served as the reference method for MTBC identification and comparative evaluation of the ICT kit. Of the 150 BACTEC culture positive isolates tested by ICT kit, 101 (67.3%) were found positive for MTBC and remaining 49 (32.7%) were considered as NTM. These results were further confirmed by IS6110 PCR that served as the reference method for detection of MTBC. H_{37} Rv reference strain was taken as a control for ICT test and IS6110 PCR. The reference strain showed the presence of MPT64 antigen band in the ICT test. Similar bands were formed in 101 of 102 MTBC isolates tested, proving 99.1 per cent sensitivity and no bands were detected in 48 (100%) NTM isolates tested, proving 100 per cent specificity of the ICT kit. Our findings show that ICT test can be used on direct culture positive specimens. It does not require any special equipment, is simple and less time-consuming. It can easily discriminate between MTBC and NTM and thus can help in appropriate management of tuberculosis.

Are all patients diagnosed with tuberculosis in Indian medical colleges referred to the RNTCP?

T.A. Quazi, S. Sarkar, G. Borgohain, A. Sreenivas, A. D. Harries, S. Srinath, K. Khan, B. Bishnu, S. Tapadar, A. Phukan, A. Kabir, V. Chaddha, D. Paul and P. Dewan. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(8)**: 1083-5.

To assess the proportion of tuberculosis (TB) patients diagnosed in three medical colleges in the states of West Bengal and Meghalaya who benefited from the services provided under the Revised National Tuberculosis Control Programme (RNTCP), a line list of patients with reports of investigations suggesting probable or confirmed TB was prepared from the records of the pathology, radiology and microbiology departments. This was compared with another line list prepared using RNTCP records. Only 150 (36%) of 420 probable or confirmed TB patients were referred to the RNTCP services. This suggests a need for more intensive supervision and training of medical college faculty.

Provider perceptions of pharmacy-initiated tuberculosis referral services in Cambodia, 2005-2010

C.A. Bell, M.T. Eang, M. Dareth, E. Rothmony, G.J. Duncan and B. Saini. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(8)**: 1086-91.

Since 2005, private pharmacies linked to the National Tuberculosis Programme (NTP) and the Municipal Health Department in Phnom Penh have referred tuberculosis (TB) symptomatic patients to public sector TB clinics. The objective was to investigate the attitudes and practices of pharmacy-initiated referral service providers in Phnom Penh from 2005 to 2010. In a qualitative study, participants were purposively selected from the register of pharmacy owners providing referral services. Discussions were conducted in Khmer by trained facilitators. Participants discussed topics relating to their experiences and participation in the referral

programme. In January 2011, 54 pharmacy owners participated in six focus group discussions held in Phnom Penh. Interpreted data showed consistency of message across all topics. The emergent themes—altruism, pragmatism and professionalism-underpinned owner commitment to programme goals. Issues associated with patient counselling, fear of infection and quality of care in public sector clinics were of concern to participants. Owners believed ongoing professional support, improved public sector patient care and media campaigns would strengthen their role. Pharmacy outlets provide further options for NTPs engaging with private sector providers. Recognising private provider needs and aspirations may be an essential component of public/private mix programmes to meet public health goals.

Community-based treatment of multidrug-resistant tuberculosis: early experience and results from Western Kenya

D. Oyieng'o, P. Park, A. Gardner, G. Kisang, L. Diero, J. Sitienei and J. Carter. *Public Health Action* 2012; **2(2)**: 38-42.

In the light of the 2010 World Health Organization estimation of 6,50,000 cases of multidrug-resistant tuberculosis (MDR-TB) globally, the need to develop, implement and scale up MDR-TB treatment programmes is clear. The need is the greatest and urgent in resource-poor countries, such as Kenya, with a high TB burden and an anticipated rise in reported cases of MDR-TB with increasing access to drug susceptibility testing. The objectives were to describe the set-up of a community-based programme, early clinical outcomes, challenges and possible solutions. The settings were the Moi Teaching and Referral Hospital (Moi Hospital) catchment areas: Western and North Rift Provinces, Kenya with the design of programme description and retrospective chart review. An MDR-TB team established a community-based programme with either home-based DOT or local facility-based DOT. Following referral, the team instituted a home visit, identified and hired a DOT worker, trained family and local health care professionals in MDR-TB care and initiated community-based MDR-TB treatment. In the first 24 months, 14 patients were referred, five died prior to initiation of treatment and one had extensively drug-resistant TB. Among eight patients who initiated community-based DOT, 87% underwent culture conversion by six months, and 75% were cured with no relapse after a median follow-up of 15.5 months. Multiple challenges were experienced, including system delays, stigma and limited funding. Despite multiple challenges, our model of an MDR-TB team that establishes a community-based treatment system encircling diagnosed cases of MDR-TB is feasible, with acceptable treatment outcomes.

Breath analysis as a potential diagnostic tool for tuberculosis

A.H.J. Kolk, J.J.B.N. van Berkel, M.M. Claassens, E. Walters, S. Kuijper, J.W. Dallinga and F.J. van Schooten. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(6)**: 777-82.

We investigated the potential of breath analysis by gas chromatography-mass spectrometry (GC-MS) to discriminate between samples collected prospectively from patients with suspected tuberculosis (TB). Samples were obtained in a TB-endemic setting in South Africa, where 28% of culture-proven TB patients had Ziehl-Neelsen (ZN) negative sputum smear. A training set of breath samples from 50 sputum culture-proven TB patients and 50 culture-negative non-TB patients was analysed using GC-MS. We used support vector machine analysis for classification of the patient samples into TB and non-TB. A classification model with seven compounds had a sensitivity of 72%, a specificity of 86% and an accuracy of 79% compared with culture. The classification model was validated with breath samples from a different set of 21 TB and 50 non-TB patients from the same area, giving a sensitivity of 62%, a specificity of 84% and an accuracy of 77%. This study shows that GC-MS breath analysis is able to differentiate between TB and non-TB breath samples even among patients with a negative ZN sputum smear but a positive culture for *Mycobacterium tuberculosis*. We conclude that breath analysis by GC-MS merits further research.

LIST OF REVIWERS, 2012

Agarwal, Nishi, *Delhi*
Agarwal, Upasna, *Delhi*
Amitava Chakrabarti, *Chandigarh*
Arora, V.K, *Delhi*
Bedi, R.S. *Patiala,*
Beena, Chennai
Behera, D. *Chandigarh*
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Singla, Neeta, *Delhi*
Surya Kant, *Lucknow*
Swaminathan, Soumya, *Chennai*
Tripathy, S.N. Cuttack
Verma, Ajay, *Delhi*

(Names are in alphabetical order)

Subject: SIXTY SEVENTH NATIONAL CONFERENCE ON TUBERCULOSIS AND CHEST DISEASES - PATNA – FEBRUARY, 2013 (NATCON 2012)

The 67th National Conference on Tuberculosis and Chest Diseases (NATCON 2012), under the joint auspices of the Tuberculosis Association of India and the Bihar Tuberculosis Association, will be held in Patna from 8th to 10th February, 2013.

Our Technical Committee has selected the following subjects for the above Conference:

1. Epidemiology of TB
2. RNTCP, HIV-TB, MDR-TB, XDR-TB
3. Pneumonia, COPD and Asthma
4. TB Diagnostics
5. Sleep-Disordered Breathing
6. Public & Private Partnership for DOTS Implementation
7. Tobacco & Lung Health
8. Pediatric Tuberculosis
9. Bird Flu (Avian Influenza)
10. Surgery in Pulmonary Tuberculosis
11. Advocacy, Communication and Social Mobilisation (ACSM)
12. Socio-behavioural studies in HIV and TB
13. Lung Health
14. TB & Diabetes

It will be appreciated if you can participate as well as kindly bring this circular to the notice of all TB and Chest Diseases Workers with you and in your area. They should let us know, whether they would like to present academic papers/posters on any of the above subjects and, if so, to forward the abstracts of the same **latest by the 31st October 2012, to the Secretary General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110001;** Telephone: 23715217; Telefax: 23711303**; E-mail:** tbassnindia@yahoo.co.in**.** The guidelines for preparation of abstracts/ slides/transparencies are given below.

For further details, you may please contact: Dr.J.N. Banavaliker, Vice-Chairman, E.mail:jnbanavaliker@yahoo.com; Mobile No.; 9810721234; Mr. Tejinder Ahluwalia, Secretary General, Mobile No.9810550888.

GUIDELINES FOR AUTHORS

A) For preparing abstracts :

1. The length of an abstract should normally not exceed 250 words, including the heading.
2. The abstract should comprise (a) objectives of the study, (b) methodology of investigation and (c) main findings. In respect of some papers, (b) and (c) may comprise the idea/ hypothesis, discussion and conclusion. Phrases like "findings will be presented at the Conference" are unhelpful.
3. If analysis is incomplete at the time, a revised abstract should be sent, at least six weeks prior to the Conference to be included in the "Programme and Summaries" for distribution among delegates.
4. An inadequate abstract may not be selected by the Programme Committee for presentation.

B) For preparing projection slides :

1. Material on the slide should be relevant, minimum, in bold letters/figures and computer composed.
2. Material should normally cover 3/5 of the available space on the slide (35mm) with margins on all sides. Professional assistance is preferable.
3. Blue on white background is better than black and white slides. For multi-colour slides, the preferable colours are red, black and green on white background.

C) For preparing posters :

1. The dimensions of your poster should not exceed 60cm horizontallyx120cm in vertical length.
2. Avoid putting too much material and text on the poster.
3. The heading should have letters at least 35mm high listing the title of the paper, authors, institution and its location.
4. Lettering for text and illustrations must be at least 10mm high, or typed clearly.
5. Divide your poster into Introduction, Objectives, Methods, Results, Discussion and Conclusions. Each of these sections should be in sequence to guide the reader through the poster.
6. The introduction should contain 3 to 5 sentences outlining essential information necessary to understand the study and why it was done.
7. The objective of the study, the questions to be asked or the hypothesis to be tested should be clearly stated in as few words as possible.
8. Outline your methods briefly.
9. Results should be presented as graphs or tables. They should be self-explanatory and therefore please provide a clear legend including symbols. You may also want to provide an interpretation of the results below each panel.
10. The discussion (if necessary) and conclusions should be succinctly stated on large type. Many viewers read this first, hence it should be easy to understand.