COMMENTARY

Tuberculosis research in India

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Tuberculosis is a highly prevalent infectious disease and globally, India is one of the 22 high-burden countries. There are diagnostics and drugs available and vaccine initiatives to control the disease. However, the need of the hour is improved Point of Care diagnostic; less toxic but cheaper drug of short duration and a new vaccine is of utmost importance. Also, other areas need to be strengthened. For these, research efforts are ongoing and Indian researchers have been involved in several diversified fields of this endeavour. This commentary deals with some of the notable research works in the recent past.

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*Mtb*), is a highly prevalent infectious disease and globally, India is one of the 22 high-burden countries and alone bears the share of 26% of global cases with TB incidence of 2.5 million as notified cases in 2011. Though about 80% of TB patients suffer from pulmonary infection (PTB), the incidence of extra-pulmonary manifestations is also high (1 in 5 patients). However, more than six lakh people in India are unaware that they suffer from TB and are spreading the disease among healthy individuals. The disease predominantly affects an economically productive age group which leads to a huge socio-economic impact. The mortality trends in India have improved through the implementation of Direct Observed Treatment Short Course (DOTS) under the Revised National Tuberculosis Control Programme (RNTCP). The vision of RNTCP for the national strategic plan (2012–2017) – reaching the unreached[1] – is a 'TB free India, through achieving universal access by provision of quality diagnosis and treatment for all TB patients in the community'. However, TB diagnostics are critical for reaching the goal of universal access even though creation of STOP TB partnership working group in 2010, for using new diagnostics. Hence, Protein Data Bank for TB and Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland have been formed. Importantly, the affordable health care-related UN Millennium Development Goals need to be achieved by the developing countries by leveraging access to and effective use of biopharmaceuticals like diagnostics, drugs and vaccines. Thus, the need of the hour is more effective development of the diagnostics, more so, a Point of Care (PoC) diagnostics as the cure starts with accurate diagnosis. Similarly, less toxic but more effective, cheaper drug of short duration and a vaccine is of utmost importance. Immunology, basic biology, host-pathogen interaction, etc. can also reveal new targets for novel drug development. Indian researchers have been involved in several diversified fields of TB research, some of the important ones are mentioned here.

TB diagnostics

The National Institute for Research in Tuberculosis, Chennai, established in 1956, is a WHO Collaborating Centre for TB Research and Training which characterizes several types of TB patients and follows them up for 5–10 years. Several clinical studies have been undertaken on drug therapies, pharmacokinetics, pharmacodynamics, drug susceptibility testing (DST), epidemiological impact studies, mortality surveys, etc. One of the groups has demonstrated the comparative assessment of three different tests, e.g. DNA sequencing, PCR-Single Strand Conformation Polymorphisms and the assay of Pha B with indirect sensitivity testing for detection of Rifampicin resistance of *Mtb*[2]. Another effort has been made for improving the sensitivity and specificity of the sputum smear quality by treating with phenol ammonium sulphate (PhAS) sedimentation[3]. Sputum is stained for AFB in the container where it has been collected by the PhAS basic fuchsin solution followed by the decolorization and retained the smears for AFB diagnosis[4]. Kumar *et al.*[5] have discovered a temperate Mycobacteriophage *Che12*, the luciferase gene of firefly has been cloned into it to make luciferase reporter phage which could infect *Mtb* for diagnosis of active infection. A cocktail of phages has been made and used to replace the antibiotics to restrict the overgrowth of other nontuberculous mycobacteria (NTM) in the sputum samples for improvement of the diagnostic capability of *Mtb*. Kumar *et al.*[6] have evaluated the role of these cocktails on sputum specimens passaged by modified Chitin and Petroff's assay for better and rapid yield of *Mtb* in LJ media. Ramachandran[7] and his group have observed better effect of a rapid yet less expensive Microscopic Observation of Drug Susceptibility (MODS) assay for measuring the DST of *Mtb* in both non-HIV TB and HIV TB from the sputum specimens of suspected PTB patients.

Shenai *et al.*[8] (at Hinduja Hospital, Mumbai) have designed an in-house Reverse Line Blot Hybridization assay for both accurate species identification of 15 *Mtb* species of clinical importance and drug resistance testing of three of the first line anti-TB drugs in *Mtb* complex. The technology has been transferred to XCyton, Bangalore for further development. An attempt is being made for diagnosing TB through volatile organic compounds present in urine and a new technology of an 'electronic nose' development by the International Center for Genetic Engineering and Biotechnology (ICGEB) supported by the Grand Challenges Canada program (GCC) and the Gates Foundation.

Tyagi's group (at All India Institute of Medical Sciences (AIIMS)) has used processed clinical samples and Universal Sample Preparation diagnostic technology to commercialize the test kit through Arbro Pharmaceuticals, New Delhi for diagnosing pulmonary and extra-pulmonary TB[9]. Haldar's group in Translational Health Science and Technology

Institute, Gurgaon has worked on new diagnostic modalities with novel TB biomarker and PoC test exploiting antigen and nucleic acid.

JALMA, Agra has signed a contract in 2007 with the Nutrapharma, USA for diagnosis of NTM through designer diagnostics NTM kits, the most predominant one as found is the *M. avium* complex in soil and water. It has patented the new efflux pumps active in the multidrug resistance (MDR), INH resistance and ofloxacin in *Mtb*. PCR-RFLP has been developed with 23S rRNA gene region restricted with HhaI enzyme for speciation of mycobacteria. Another researcher has extensively worked on epidemiology and pathogenesis of TB; also molecular insights into spread of MDR TB in India[10,11].

The group led by Hasnain (IIT, Delhi) has focused on the PE/PPE proteins involved as diagnostic marker, cell signalling, host–pathogen interaction, molecular mimicry, etc.[12]. The predominance of the less virulent, ancestral strains of *Mtb* circulating in India; India's first whole genome sequencing of *Mtb*; the immunomodulatory role and biomedical importance of soil Mw, renamed as MIP/*Mycobacterium indicus pranii* have also been studied by him.

Several indigenous or imported serological tests have been performed for rapid diagnosis in most of the private healthcare sectors in India. However, no perfect test has come up with excellent performance and recently, WHO, Geneva has issued a negative policy[13] on the use of any serological test. However, it does not imply banning of research on development of any novel serological test.

Cepheid, USA through FIND, has launched GeneXpert MTB/RIF, to diagnose TB and MDR TB which takes two hours for results and seems better than the microscopic evaluation. This is being evaluated in India. STOP TB Partnership has mediated a clinical trial of Signature Mapping Medical Sciences, USA developed automated TB detection system (TBDx) for Auramine–O stained slides through Apollo Hospitals.

Bigtec Laboratories, Bangalore and the Tulip Group, Goa have jointly developed a hand held battery-powered and cheap polymerase chain reaction (PCR) test to be used in peripheral settings, through CSIR's NMITLI programme and GCC. A PCR test has been developed by XCyton in partnership with a public research group funded by NMITLI programme. A multiplex PoC diagnostic platform based on microfluidics has been launched by Achira Labs, Bangalore, which has explored an innovative technology of handwoven silk 'chips' supported by GCC. TB-specific glycolipids have been explored by Jiwaji University in partnership with Bisen Biotech, Gwalior to develop a TB test[9].

TB drug development

Efforts in India have included the sudoterb by Lupin Ltd, Mumbai for which the Phase I Trial has been cleared and the next phase to assess the bactericidal effect has begun. The safety trial of benzothiazinone by AstraZeneca, Bangalore has also started. The Central Drug Research Institute, Lucknow has developed inhalable microparticles for PTB. One very new and innovative example can be cited as the Open Source Drug Discovery (OSDD), by CSIR as a Consortium acting as a global translational platform for drug discovery. Currently, through the TB Drug Alliance effort, India has entered into a global clinical trial for three drugs namely Sirturo/ bedaquiline, PA 824 and Delaminid for treating both drug-sensitive and drug-resistant TB. AIIMS and NIRT have been doing trial for Bedaquiline.

A research group (Rajiv Gandhi Institute of Chest Diseases, Bangalore) has studied the drug-resistance and treatment outcomes in the 224 confirmed MDR-PTB patients till 2010. They have reported that majority of patients are resistant against all four first-line drugs. However, others develop variable resistance against different drugs. Hence, the treatment pattern has to be decided based on the drug resistance[14].

Vaccine initiative

Vijaya's group (Indian Institute of Science, Bangalore) has explored a new promoter in the *Mtb* genome which functioned efficiently in *E. coli* to construct a vector. This has been used to drive expression of heterologous genes in *Mtb* and in Bacillus Calmette–Guérin (BCG) which can be a promising BCG-based multivalent vaccine. They have found antigens coded by several novel genes of *Mtb* that could elicit human T cell responses of the TH1 phenotype which enable protection against an experimental challenge in the guineapig animal model. These vaccine candidates are under clinical trials. They have used Green Fluorescent Protein-tagged genes inserted into the *Mtb* genome and monitored movement of these proteins inside the infected macrophages. Simultaneously, a group in Delhi University, South Campus has developed expression of *Mtb* antigens in BCG to develop candidate recombinant BCG vaccine (rBCG85C) and candidate DNA vaccine and evaluated them against aerosol infection of *Mtb* in guineapigs by heterologous prime boost approach[15]. These have been expressed sustained and better protection than BCG and recommended for human clinical trials. Also, they are engaged in identification, validation and application of new drug targets for TB as well as structure and function analysis of mycobacterial transcription signals and gene expression. A similar approach on rBCG85C bovine TB vaccine development has been carried out jointly with AIIMS and Biovet Pvt Ltd, Karnataka.

Basic biology

Nagaraja's group (IISc, Bangalore) has done extensive work on mycobacterial DNA gyrase and topoisomerase I involved in regulating the super coiling events in the cells[16]. Balaji and group has studied the signalling mechanisms involved in macrophages/dendritic cells when infected with pathogenic mycobacteria. Different survival strategies exhibited by *Mtb* and also the PE/PPE family antigens of *Mtb* have been functionally characterized by him. A group led by Muniyappa has done several proteomics study on RuvAB proteins of *Mtb*; role of UvrD1 and UvrA proteins on suppression of DNA strand exchange, etc.

Studies are in progress at the Institute of Genomics and Integrated Biology, New Delhi on Chemical Biology of *Mtb* by exploiting the metabolic pathways and mechanisms involved which generates molecular diversity responsible for the virulence and pathogenicity of the bacteria. Rao and his group (ICGEB) have been experimenting the signal transduction pathway of *Mtb*, more so, the details of secreted antigen namely MTSA-10 modulating macrophage function by redox regulation of phosphatases. Analysis of genetic polymorphism from

Mtb isolates from patients of different geographic regions revealed that RD1 region is frequently present in Indian isolates and RD9 region is well conserved. These results indicated that Indian *Mtb* strains are more ancient[17]. They have also studied molecular epidemiology of TB with the help of a systems biology approach[18]. It is proven that when human being stay in long, frequent, or close contact with TB patients, they face a high risk of contracting the disease which has a 22% infection rate[18]. Das *et al.*[19] have recently demonstrated the survival of dormant *Mtb* in the $CD271^{+}$/$CD45^{-}$ human bone marrow Mesenchymal Stem Cells in *in vitro* culture, both in mouse and a human model.

The National Tuberculosis Institute, Bangalore has worked on surveillance of drug resistance and TB control strategies in sync with regional and national programme delivery.

For a killer disease like TB, the pressure to treat as many people as possible, with limited resources is of prime importance. Though enough research has been done on TB, special efforts are still needed to make the DOTS program more successful. Multiple diagnostic samples loading, sophisticated imaging technologies and sample handling procedure, cost effective technologies, regulatory policies, etc. will definitely hasten up the process.

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