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Recent advances in management of tuberculosis in infants

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Abstract. – The global tuberculosis (TB) incidence estimated by WHO is found to be 8.6 million people. Moreover, the highest TB burden worldwide is found in Asian and African countries. The disease is more prevalent in infants due to their immature immune systems. Despite this, the available diagnostic tools pose a challenge due to paucibacillary nature of the disease and difficulty in obtaining specimens. The present review article discusses the important and upcoming advancements in the management of above pathological state. The article will enlighten the new vaccinations for TB in the pipeline. Moreover, new upcoming approaches involving system biology and gene expression profiling for efficient supervision of the disease will also be highlighted.

Key Words:
Tuberculosis, Infants, New vaccines, Gene profiling, System biology.

Introduction

Tuberculosis (TB) is an infectious airborne disease caused by infection with the bacterium *Mycobacterium tuberculosis (M.tb).* Although TB could affect almost every organ of the body, *M.tb* primarily infects lungs[1,2]. It is transmitted to an uninfected person by inhalation of air infected by a bacterium from an infected person's cough. However, not every infected person tends to progress to TB disease. Only about 10% of infected people progress to disease, but this risk is increased up to 20 fold in HIV-positive individuals[3,4]. Further, infants and young children (less than 4 years old) have an immature immune system compared to older children and adults[5,6], they tend to be more susceptible to TB disease[7].

The basic protocol to tackle TB included diagnosis of active disease followed by treatment and vaccination for future threats. For diagnosis of disease, a wide range of assays is used which included sputum microscopy, culture, X-ray, PCR, epidemiological scoring and nucleic acid amplification technology (NAAT) method (Gene Xpert MTB/RIF assay)[8-10,11]. Bacteriological confirmation of *M.tb* presence in sputum by microscopy and/or culture is the most definitive way of diagnosing TB. However, it has a limitation for infants as sputum samples are difficult to collect in young patients and the number of bacilli is also very scarce[12]. So, sputum induction and gastric lavage are in use for collection of sputum samples from young patients. Induced sputum is a simple and non-invasive process that basically promotes coughing up of sputum[13]. On the other hand, gastric lavage involves the insertion of a tube down through the nose or mouth for suction of small volumes of normal saline[14]. Chest X-rays are also commonly used as the first method to diagnose active TB disease. Misdiagnosis by X-rays is common in the cases where there is a lack of technical expertise or in the patients affected by HIV[15,16]. The latest diagnostic test endorsed by WHO for TB diagnosis is the Gene Xpert MTB/RIF assay that detects *M.tb* DNA in sputum samples, with a read out in less than 2 hours[17]. However, the major limitations of this test are cost and intensive maintenance[18,19]. The present review article shall put light on the latest advancements being made in the field by the medical physicians in order to overcome existing limitations for the efficient management of tuberculosis in young infants.

Current Treatment Modalities for Tuberculosis

The current treatment schemes have been summarized in Table I. Further, there are more than 22 drugs available to treat TB, which are in use in different combinations based on the circumstance (Table II). Drugs used to treat TB can be combined into the first line (used to treat new TB cases or patients unexposed to prior TB treatment),

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second or third line regimens (used for the treatment of drug-resistant TB)[20]. TB treatment regimen is similar between children and adults but different for latent infection and active disease[21]. Latent TB infection could be treated with the first line drug Isoniazid for 6 months or a combination of Isoniazid and Rifampicin for 3 months[22]. However, treatment of LTBI in high burden settings is limited only to children under 5 years of age and HIV-infected adults. Patients with active TB disease could be treated with a combination of multiple first-line drugs for 6 months in two phases[23]. Non-compliance with the treatment program might lead to the emergence of resistant strains of TB[24]. Treatment of multi-drug resistant (MDR) TB is more difficult in comparison to drug-susceptible TB. XDR M.tb is resistant to the first line as well as second line drugs. Therefore, XDR M.tb is treated by the third line of drugs and these drugs are often less effective, more toxic and expensive[25].

The vaccination approach has also shown some positive results against TB and the only licensed vaccine available against TB is Bacille Calmette-Guerin (BCG)[26]. This vaccine is effective in the prevention of severe forms of TB in children[27]. However, this vaccine has been reported to show variable efficacy in protection against pulmonary disease across different groups[28,29]. So, there is an urgent need for the development of new and better vaccines against TB. Moreover, due to its efficacy in infants, BCG is still commonly used in high TB burden countries and even in countries where protective efficacy is negligible, especially in babies. Further, prominent reasons for the variable efficacy of BCG included (i) Different BCG strains; (ii) Host genetic variation; (iii) Pre-exposure to non-tuberculous mycobacteria (NTM); (iv) Interference by parasitic infections.

New Upcoming Vaccination Approaches

There are around 14 TB vaccine candidates currently in clinical trials (Table III). Moreover, 35 vaccine candidates are at preclinical stage, and 21 are next generation vaccines in the discovery phase[30]. Next generation vaccines are vaccines that are in the research and development stage with some preclinical evidence of conferred protection. There are 3 broad categories of these vaccinations, which are discussed below.

Live Attenuated or Recombinant vaccines

These vaccines are designed to replace BCG as prime vaccines. A live attenuated vaccine is a vaccine whose virulence has been weakened by deletion of certain genes but could still induce a cellular immune response. An example of such a vaccine in clinical trials is MTBVAC, which is a live attenuated *M.tb* vaccine[31]. This vaccine has been observed to induce an immune response that is long lasting in mice in comparison to that of BCG. On the other hand, another live recombinant vaccine comprised of modified BCG and is able to over-express key *M.tb* antigens. This vaccine is called VPM1002 and has been constructed by knocking out of the urease gene from BCG and insertion of listeriolysin gene from *Listeria monocytogenes,* thereby allowing the recombinant

Table I. Treatment schemes for TB in infants.

Sr. No.	Name of the treatment	Short description
1.	Therapeutic treatment	Use of first line, second line and third line drugs depending upon the severity of TB
2.	Vaccination	Use of live attenuated bacteria for developing immunity against TB Different forms are available 1. Live attenuate or recombinant 2. Viral vector based 3. Adjuvanted subunit vaccines 4. Whole Cell based
3.	Upcoming System biology approach	It explores sequencing of deoxyribonucleic acid (DNA), including intra organism cell specific variations e.g. Telomere variation that helps in identification of cell specific gene expression regulating factors.
4.	Gene expression profiling	It define physiological states and infer phenotypes based on gene expression patterns specific for a particular disease like TB

Table II. Current drugs in use for TB.

First line drugs (Oral)	Second line (Injectable drugs)	Second line (Fluoroquinolones)	Second line agents	Third line New drugs with an unclear role in TB
Isoniazid	Kanamycin	Levofloxacin	Para-aminosalicylic acid	Clofazimine
Rifampicin	Amikacin	Moxifloxacin	Cycloserine	Linezolid
Pyrazinamide	Capreomycin	Ofloxacin	Terizidone	Amoxicillin
Ethambutol	Streptomycin		Thionamide	Thioacetazone
Streptomycin			Prothionamide	Clarithromycin

Table III. TB vaccine development.

Agent	Strategy	Type	Sponsors	Status
M. indicus pranii	Immunotherapeutic	Whole cell M. indicus pranii	Department of Biotechnology Cadila Pharamaceuticals	Phase III
M. Vaccae	Immunotherapeutic	Whole cell M. Vaccae	AnHui Longcom	Phase III pending
MVA85A/ Aeras-485	Prime Boost	Viral Vector	Oxford University, Aeras	Phase IIb
M 72 + AS01	Prime Boost	Adjuvanted subunit	GSK, Aeras	Phaset IIb
Crucell Ad 35/Aeras-402	Prime Boost	Viral vector	Crucell, Aeras	Phase II
VPM 1002	Prime	Live recombinant rBCG	Max Planck Institute of Infection Biology	Phase IIa
RUTI	Immunotherapeutic	Fragmented MTB	Archivel Farma	Phase IIa
Hybrid 1 + IC31	Prime boost	Adjuvanted subunit	Statens Serum Institute, Intercell, Europoean and Developing Countries	Phase IIa
Hybrid 56 + IC31	Prime boost	Adjuvanted subunit	SSI, Aeras	Phase IIa
Hydbrid 4 IC31/Aeras-404	Prime boost	Adjuvanted subunit	Sanofi Pasteur, Aeras	Phase IIa
ID93 + GLA-SE	Prime boost	Adjuvanted subunit	Infectious Disease Research Institute, Aeras	Phase I
Ad5Ag85A	Prime boost	Viral vector	McMaster University, CanSino	Phase I
MTBVAC	Prime	Live genetically attenuated MTB	University of Zaragoza, TBVI	Phase I
Dar-901	Prime Boost	Whole Cell M. Vaccae	Geisel School of Medicine, Dartmouth University	Phase I pending

BCG to escape the phagosome[32-34]. Another live recombinant vaccine that reached phase I clinical testing in 2004 is rBCG30, but is in developmental stage[35].

Viral-vectored Vaccines

These vaccines are designed by modification of non-replicating viruses, which serve as a transport of M.tb DNA into human cells. Upon entry into cells, the M.tb DNA is transcribed into proteins, and these proteins could then induce immune responses[36]. These vaccines have mainly been used in prime-boost strategies to boost the immune response induced by BCG. MVA85A, Crucell AD35, and Ad5Ag85A are examples of viral vectored vaccines.

Adjuvanted Subunit Vaccines

These vaccines are designed by fusion of different *M.tb* proteins into immunogens, which are then formulated with specific adjuvants. In the field of vaccinology or immunology, adjuvants are agents that are added to vaccines to provoke the immune system to respond to a particular antigen but do not confer immunity themselves[37]. The most advanced of these vaccines is M72, which contained the *M.tb* proteins 32A and 39A in a fusion protein and is formulated in the adjuvant AS01. This vaccine has been shown to induce good Th1 and Th17 responses in a population of *M.tb* infected individuals in South Africa[38]. In the same study, there was evidence suggestive of the fact that this vaccine might boost T cell populations primed by natural infection with *M.tb*.

Whole Cell or Fragmented Mycobacterium Vaccines

These vaccines are made up of replication-deficient, inactivated whole cell or fragmented mycobacteria. In combination with chemotherapy, these vaccines might be therapeutic as they work to improve the treatment of either active disease or LTBI. Examples of such vaccines include RUTI, Dar 901, *M. Vaccae*, *M. indicus pranii*.

Major limitation of Vaccination Approach

The major limitations of vaccination approach against TB are the inter-individual variations in vaccine-induced immune responses. Variability in immune responses is due to host factors including genetic factors, environmental factors like demographic distribution, co-infections, pre-exposure to vaccine antigens and vaccine delivery routes. Polymorphisms in key immune response genes have been also associated with heterogeneity of vaccine-induced responses including BCG[39,40]. Pre-exposure to vaccine antigens and/or vectors is another co-factor for heterogeneity in vaccine-induced responses and has been observed in various vector-based vaccines too[41]. Age and gender of the patient also contribute significantly towards vaccine-induced immunogenicity or efficacy[42,43]. BCG has been shown to induce variable response in infants vaccinated at birth compared to later time points[44]. Co-infection with bacteria, viruses as well as parasites has effects on the human immune system and this could consequently affect the immunogenicity of several vaccines[45].

System Biology in Tuberculosis – an upcoming Modality

Systems biology is an emerging research strategy that focuses on complex interactions within biological systems using a holistic approach, rather than a reductionist approach. The overall aim of systems biology is to understand the dynamic aspects of networks[46]. To understand complex biological systems, integration of experimental as well as computational research is necessary[47]. Systems biology is often studied by monitoring relevant pathways after perturbation of biological systems[48]. This is followed by integration of large data sets to formulate mathematical models that describe the system's structure and its response to perturbations[48]. System biology is being utilized in current research to explore sequencing of deoxyribonucleic acid (DNA), including intra organism cell specific variations *e.g.* Telomere variation. It also helps in identification of cell specific gene expression regulating factors that are not empirically coded in the genomic sequence *e.g.* DNA methylation. Moreover, the study of variation in phenotypes as they change in the tuberculosis progression and over the lifespan is also being studied with the help of system biology approach.

Gene Expression Profiling – new way to Tackle TB

The measurement of the activity (expression) of thousands of genes at once to form a global picture of cellular functions is known as gene expression profiling[49]. The gene expression provides the global picture possible in a single experiment. This approach assumed that genes that share a common pattern of expression under certain conditions are functionally related to each other. Moreover, any change in these gene expression pathways reflects the physiological changes in a cell related to the pathological state concerned. Therefore, this approach makes it possible to define physiological states and infer phenotypes based on gene expression patterns specific for a particular disease like TB[50]. Gene expression profiling usually involves the use of high-throughput techniques like serial analysis of gene expression (SAGE), massively parallel signature sequencing (MPSS), oligonucleotide arrays, cDNA microarrays and most recently RNA sequencing (RNA-Seq) (51). Microarrays are currently the most widely used technique as it offers identification of relevant genes and expression pathways that could be further investigated in future studies as potential biomarkers as well as specific drug targets.

Conclusions

A lot of researchers are involved intensively to explore all the possible advancements to tackle this pathological state right from infant stage. The use of latest technical approaches like system biology along with gene expression profiling arrays shall definitely bring highly specific and equally efficient solution for tuberculosis management in infants.

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