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# AN UPDATE ON NOVEL TECHNOLOGIES, *IN-VITRO* AND *IN-VIVO* MODELS FOR TUBERCULOSIS AND TUBERCULOUS MENINGITIS

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**ABSTRACT:** Tuberculosis is a bacterial infection which is caused by the bacteria *Mycobacterium tuberculosis*. It is next to AIDS and causes morbidity and mortality. These bacteria can stay in the host's body for decades. In this article we have mentioned about the pathogenesis of pulmonary tuberculosis followed by the pathogenesis of tuberculous meningitis wherein the bacteria first enter to the lungs which then form into macrophages, these macrophages form into granulomas which get ruptured and enter the brain and then enter the subarachnoid space causing tuberculous meningitis. The current treatment methods available, their dosage forms and penetrance into the CSF is also mentioned. Usually, the first line drugs are used in combination to treat the disease but in the case of resistance second-line drugs are used. The duration of the treatment depends on the severity of the disease. Even though, there are many drugs available to treat the disease the amount of drug entering the brain is minimal due to the presence of the blood-brain barrier (BBB). So, due to this reason many new technologies like niosomes, liposomes, nanoemulsions, microspheres, *etc*. are being used. Hence, in this paper, we are explaining about tuberculosis as well as tuberculous meningitis and its possible strategies for overcoming the problems associated with the failure of the treatment.

**INTRODUCTION:** Tuberculosis is the disease which is caused by the bacteria *Mycobacterium tuberculosis*, but there are various genotypes of *M. tuberculosis* bacteria. Among them, the Beijing family of *M. tuberculosis* is considered the most virulent [1]. It is also associated with drug resistance. Robert Koch received the prize in 1882 for the discovery of the bacterium *Mycobacterium tuberculosis*. This bacterium can stay in the host for decades [2].

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According to the global reports around 8.6 million new cases and 1.3 million deaths occur annually. This occurs despite the presence of the vaccine which is injected almost to every individual. Recent technology has shown advances in the DNA sequencing, and its impact on the tuberculous genomics has been found out [3].

Tuberculosis in children and infants is caused due to the exposure in the household with active TB. They have an increased risk of infection. Infants have around 50-60% more chances of getting the disease. Because of the low immunity and the virulence of the bacteria, there are many chances of mortality in children when compared to adults. It is caused even when there are less amount of bacteria present. It is more prominent in the children below the age of two years. 45-15% of extra-pulmonary

tuberculosis cases are related to the CNS tuberculosis [5, 58, 60]. In this review, we discuss elaborately the TB and TB meningitis, and more emphasis are given to complications of TB meningitis, *in-vitro* and *in-vivo* models to assess the efficacy of the drugs

## DISCUSSION:

**1. Incubation Period:** The incubation period is the time at which the infection has started to the onset of the disease. The incubation period for tuberculosis varies from individual to individual since some individuals have incubation periods in decades. It also depends upon whether the incubation period is considered prospectively or retrospectively [6]. At least 10 months of treatment is required to cure the disease [7]. The bacteria take around 12 weeks to produce the disease.

**2. The lifecycle of *Mycobacterium tuberculosis*: [3]** The endurance of the bacteria in the human population is dependent on the consecutive cycles of the tuberculosis infection. Then the bacteria get reactivated, and it gets transmitted into the host. The presence of bacteria in a single host leads to the exposure of the bacteria to the cul-de-sacs which then leads to the elimination of the bacteria from the host. Then it prevents the transmission of the disease to the other host. The cycle of the bacterium in a particular individual is not defined for a certain period.

The bacterial strains can enhance the increased activity of the DNA repair called recombination of the DNA. Then these DNA undergoes sampling called conjugation. Horizontal gene transfer was the method followed which led to the *Mycobacterium tuberculosis* infection which was present in the humans. When compared to other Mycobacterial species *Mycobacterium tuberculosis* does not have plasmids. So, it has diverse features when compared to the other species of the *Mycobacterium bacilli*. The adaptation of the *Mycobacterium tuberculosis* bacteria is different.

**3. Pathogenesis: [3]** Primarily the *Mycobacterium tuberculosis* enters by the nasal route, *i.e.*, through the nose. It then enters into the lungs, and the bacilli get accumulated into the alveoli. To get infected there should be the presence of many numbers of host and should be present in the bacteria for a certain period before it gets transmitted to the new host. Before the bacteria enter into the alveoli, it should be resistant and should be able to cross the barriers and then it can enter into the alveoli [3]. The bacilli are presented on the cell surface and are transferred through the lymph nodes, and the Mtb antigens enter the T-cells. The secretion of the cytokines causes the proliferation of the CD4T cells and leads to the secretion of interferons. This leads to the activation of the macrophages. Primary tuberculosis or latent tuberculosis may be caused by the Mycobacterium tuberculosis bacteria [4, 55].

During inhalation, the bacteria are exposed to many proteins and peptides which exhibit bactericidal and immunomodulatory effects. They produce many cells, *i.e.*, neutrophils, monocytes, macrophages, T-cells and epithelial cells. Neutrophils are abundantly present in the adults suffering from pulmonary tuberculosis, and the bacteria are present in an active state of division. This leads to the formation of granuloma because of the increased presence of the cytokines and chemokines. Then the bacteria are engulfed by the macrophages and inhibit the synthesis of the cytokines and chemokines which helps in producing a fixed immune response.

Usually, children have almost the same number of macrophages in 24-48 h. They have less function due to less macrophage phagocytosis. Dendrites are important for producing the antigen-specific t-cell response. Dendrimers are affected by *M. tuberculosis* by c-type lectin and CR3 mannose receptor. Then the activated dendrites are moved to the lymph nodes which gives modifying immune response [4, 55].

**CD4T-Cells:** Cell immunity is essential for the control of Mycobacterium tuberculosis infection. Usually, CD4 T- cells produce cytokines which comprise INFɤ, IL 2 and TNFα. INFɤ is essential for the activation of macrophages and to prevent tuberculosis. HIV infection leads to increased chances of tuberculosis infection and severe TB disease. When compared to adults children have many numbers of CD4-T cells, but children are more prone to the infection when compared to adults. The production of these cells led to the reduced development of Interferon-gamma release assays.

**CD8T-Cells:** CD8-T cells exhibit a cytotoxic effect and show microbial perforins and granulysin. Granulysin is produced by CD8T cells and natural killer cells which help kill the extracellular and intracellular mycobacteria.

**Natural Killer Cells:** Most of the natural killer cells are cytotoxic which produces lysin to kill the cells. In adults with tuberculosis, there was a decrease in the number of natural killer cells.

**B Cells and Antibody:** By antigen presentation, co-stimulation and cytokine production B cells are recognized as immunomodulators. Isotypes of the antibody which are used in immune response effects the activation or inhibition and also influence the outcome of the disease. Then at least one bacterium should be able to produce the infection in the host and should also overcome the immune defense mechanism of the host which leads to replication of the bacteria and produces a new cycle of infection. To produce the infection in the new host, the bacteria must replicate to get a number which is needed to produce infection in a new host. Approximately 10 lakhs of bacteria are required to produce the infection in a single lesion.

During the diseased condition due to the presence of the immune response, there will be a depletion of around 100 bacilli per lesion. When the carrying capacity of the lesions for the bacteria increase, it leads to a condition called as tuberculosis pneumonia. This condition occurs very rarely. The above-described situations imply that the mutations generated during the infection of the host achieve low frequencies in the population. So, fewer resistance mutations are selected and are tested in TB control programmes. So, by these modern techniques, it is found out that the virulence of the strains of the bacteria has been reduced in the population.

**How the Hypervirulent Strains Spread the Disease?** It has been found that the *Mycobacterium tuberculosis* infects the immune competent individuals. When the infection and disease occur due to the decreased immunity the individuals who are HIV positive patients are the poor transmitters of the tuberculosis disease. The ability of the virulence in the pathogen is directly related to the transmission of the disease. Due to the diversity in the strains and the competition between the genotypes of the tuberculosis bacterium leads to a virulent drug resistance which is cost effective. The Beijing family of the tuberculosis bacteria is hypervirulent and is drug resistant.

It may affect any part of the body, *i.e.* brain, liver, kidney spinal cord, genitals, *etc.* [8] It primarily affects the lungs, but when it affects the brain it is termed as tuberculous meningitis. It also causes morbidity and mortality in patients [9]. Meningitis may occur in people due to the breaking of the present tubercule with people suffering from effective cell-mediated immune response [9]. It is most commonly affected in pediatrics and HIV positive patients. Meningitis accounts for 1% of all cases of tuberculosis [10].

**4. Complications of Tuberculosis:** [11]

1. Acute and sub-acute complications
2. Chronic complications

**1a) Tuberculosis Sepsis and Acute Respiratory Failures:** In a few patients suffering from tuberculosis, they may have sepsis or sepsis shock. It may also cause acute lung injury which may be fatal and may lead to morbidity and mortality. If there is a delay in the TB therapy given to these patients there are very fewer chances of survival.

**1b) Immense Hemoptysis:** This condition occurs very rarely, but it leads to a serious condition. It may be caused due to the erosion of TB into the bronchial circulation or pulmonary circulation.

**2a) Architectural Compromise of Lung Parenchyma:** In pulmonary TB damage occurs in the lung parenchyma which may lead to complications in spite of the treatment given for TB. It may lead to a decrease in the pulmonary function. This extreme damage in the pulmonary function may lead to the damage to the specific lung.

**2b) Mycetoma:** The TB lesions which are present in the lungs are infected by fungi like *Aspergillus*. It leads to the development of fungal balls called mycetomas. The only measure to treat this is surgery.

**5. Complications of Tuberculous Meningitis:** [54]
These can be divided into two types:

**I. Complications Caused Due to Medications:** These complications include hepatotoxicity, peripheral neuropathy, rashes, retinopathy, and neutropenic sepsis.

These complications do not last for a longer period and can be treated easily. These complications do not cause any problem to the patient when treated in the earlier stages.

**II. Long-term Complications:** These complications include cognitive impairment, epilepsy, severe hearing loss, hypothalamic dysfunction, and dysphasia. These complications last for a longer period and the treatment also continues for a longer period. The treatment may last for a few months to years or may last for the lifetime of the individual

## 6. Mechanism: [12]

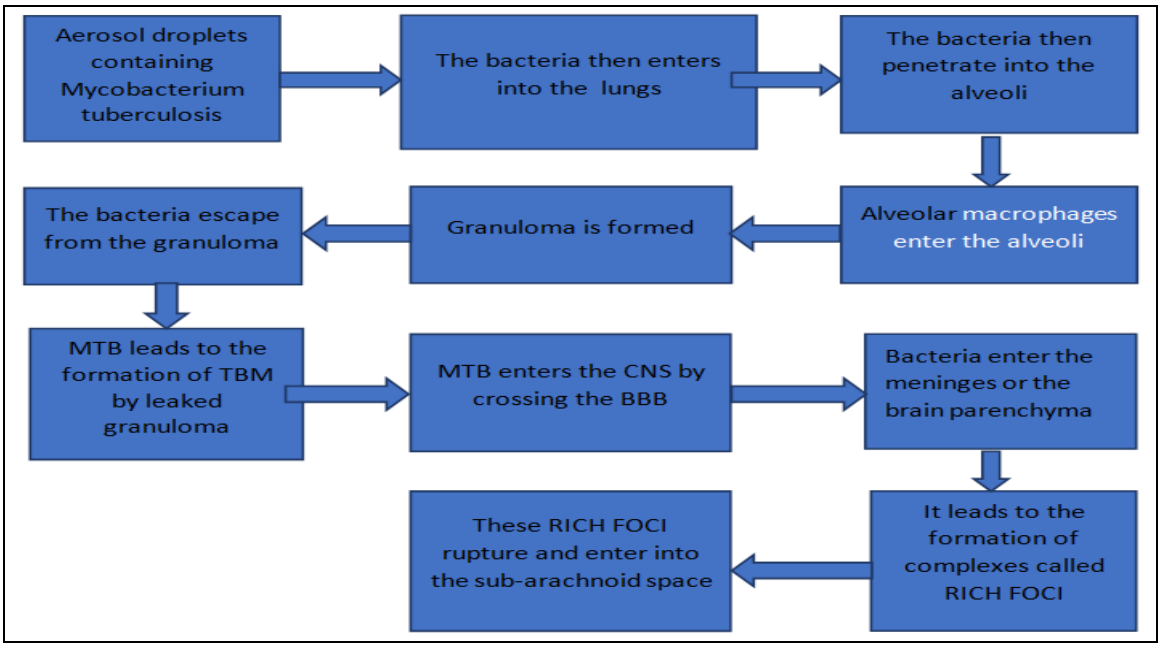


**FIG. 1: IT INDICATES THE MECHANISM OF THE TRANSPORT OF *MYCOBACTERIUM TUBERCULOSIS* FROM THE LUNGS TO THE BRAIN LEADING TO TUBERCULOUS MENINGITIS**

**7. Pathogenesis of TBM:** *Mycobacterium tuberculosis* is aerobic, non-motile, non-spore forming acid-fast *Bacillus* that infects human's primarily [10]. The doubling time of this bacillus is slow, and it takes around 15-20 h. The bacteria take around a few weeks to grow in the culture medium [10]. The bacteria enter through the nasal route through inhalation and then enters into the alveoli. Then they form granulomas which then enter into the brain by crossing the BBB [12].

Primarily the CNS tuberculosis is called as the Tuberculous meningitis (TBM) and sometimes it also mentioned as tuberculous encephalitis, intra-cranial tuberculoma or a tuberculous brain abscess. In a study conducted by Rich and Mc Cordck on guinea pig and rabbits, bacilli were to be directly injected into the CNS to produce TBM. It was observed that the bacilli entered into the subarachnoid space and helped to induce meningitis. Donald *et al*., after examining the previous studies said that the mode of entry does not explain how the TBM is caused. It was also found that disseminated TB plays a major role in TBM development in children [10].

The tuberculosis bacteria are transmitted *via* the nasal route. It undergoes phagocytosis of MTB by alveolar macrophages which are present in the alveoli. Then the granuloma is formed in the lungs; this granuloma occurs due to the responses of cellular cytokines networks [9]. Due to this the activation of type 1 T-helper cell-mediated immune response occurs. This immune response leads to the formation of granuloma. The bacteria spread to the most oxygenated regions of the body, *i.e*., even to the brain [10]. In TBM cytokines and chemokines are continuously released from the host cellular response. TNFα is the main cytokine in the inflammatory response.

It is also a major determinant in the rabbit model of meningitis. It also helps the host in granuloma formation. Another cytokine Metalloproteinase-9 plays an important role in TBM pathogenesis. It acts by increasing the degradation of BBB. Host immune factor is the major factor that leads to the formation of TBM. Then the MTB escapes from the granuloma which may usually be seen in latent TB patients. This MTB may cause tuberculous meningitis by escalating the bacteria from the lungs or it may due to the leaked granuloma which is then passed through a regional lymph node.

After spreading through the blood circulation, the bacteria enter the CNS by crossing the BBB. These bacteria cross the BBB by two mechanisms, *i.e.*, transcellular and paracellular or the infected phagocytes may migrate through Trojan horse mechanism. These bacteria enter the meninges or brain parenchyma by forming a subependymal primary complex. These complexes are termed as Rich-foci. These Rich-foci increase in size, rupture and gets spread or enters into the subarachnoid space [9]. CNS production of TNFα leads to altered BBB permeability and CNS leucocytosis and leads to TBM progression [10].

**8. Drugs Used in the Treatment of Tuberculosis Meningitis:** The primary treatment for tuberculous meningitis lies in the first line drugs. Among these drugs, isoniazid lies as a backbone due to its penetration through the BBB. Rifampicin and ethambutol have less penetration when compared to isoniazid but are effective when used in combination [10]. Streptomycin was the first drug used in the treatment of tuberculosis [13]. For the first two months of treatment, a combination of 4 drugs isoniazid, rifampicin, pyrazinamide, and ethambutol is used followed by the combination of isoniazid and rifampicin for 10 months [13, 14].

**TABLE 1: DRUGS USED IN THE TREATMENT OF TUBERCULOSIS MENINGITIS [10, S57]**

| Drugs | Recommended dose | Recommended duration | CSF penetrance |
|---|---|---|---|
| First Line Drugs | | | |
| Isoniazid | Max 0.3 g | 1 year | 80-90% |
| Rifampicin | Max 0.6 g | 1 year | 10-20% |
| Pyrazinamide | 0.025 g/kg | 2 months | 90-100% |
| Ethambutol | 0.015 g/kg | 2 months | 20-30% |
| Second Line Drugs | | | |
| Levofloxacin | 0.010-0.015 g/kg | For the whole period of treatment | 70-80% |
| Moxifloxacin | 0.4 g | For the whole period of treatment | 70-80% |
| Amikacin | 0.015 g/kg | Intensive phase only | 10-20% |
| Kanamycin | 0.015 g/kg | Intensive phase only | 10-20% |
| Capreomycin | 0.015 g/kg | Intensive phase only | No data |
| Ethionamide | 0.015-0.020 g/kg | For whole period of treatment | 80-90% |
| Cycloserine | 0.010-0.015 g/kg | For whole period of treatment | 80-90% |
| Linezolid | 0.6 mg | For whole period of treatment | 30-70% |

Isoniazid is the first primary drug used in the treatment of TB due to its higher penetration through the BBB. Rifampicin is used in combination due to the higher mortality rate of the tuberculosis bacteria. Pyrazinamide can easily penetrate the BBB and is the main drug which helps in reducing the period for the treatment. If there is an intolerance for pyrazinamide, then the treatment is to be continued for 18 months. Fluoroquinolones, *i.e.,* levofloxacin & moxifloxacin also have greater penetration into the brain and can easily cross the BBB. In the case of tuberculous meningitis, corticosteroids are given in combination with the first line drugs to provide effective treatment and reduce morbidity and mortality [14].

**9. Multidrug-Resistant Tuberculosis:** There are no controlled trials for the treatment of MDR TB [14]. Multidrug-resistant TB refers to the resistance to drugs like isoniazid and rifampicin [14, 15]. It is caused because of the irregular intake of drugs by patients during the period of treatment [16]. It is the most common factor diagnosed in most patients. It's a difficult task to suspect MDR TB. Most of the mortality cases are due to MDR TB which is not diagnosed. These patients are treated with second-line drugs which have more side effects when compared to the first line drugs [15]. This treatment is very expensive and toxic. The second line drugs used have severe side effects. The treatment continues up to 2 years, and some drugs are to be given parenterally [2].

**10. Extensively Drug-Resistant Tuberculosis:** XDR-TB causes maximum risk to the health of patients. It causes resistance to fluoroquinolones and injectable second-line drugs. So, the treatment of XDR-TB is difficult. It was first diagnosed in South Africa and the patients infected died within a few months. It was estimated that around 5% of MDR-TB cases are XDR-TB cases [2, 59].

**11. Novel Technologies Used in the Treatment of Tuberculosis:** Many drugs are available for the treatment of tuberculosis, but there is a scarcity for the availability of an effective delivery system. So, the nanotechnological based treatment is adapted to decrease the dose, controlled release, sustained release and due to which there will be an increase in bioavailability of the drug [2].

**Nanoparticles Based Delivery:**

**Oral Delivery:** The oral delivery of nanoparticles is preferred because of the sustained release of the drugs and its increased stability. The dosage regimen of the drugs can be reduced because the drug shows its action at the affected site for a long period when compared to the conventional form of the drug. The drugs loaded in nanoparticles showed action for 6-8 days [2].

**Ligand Conjugated Oral Delivery:** The attachment of the ligand to the drugs loaded in nanoparticles increased the contact of the formulation to the mucous membranes due to which increased the bioavailability of the drugs. It was helpful for oral delivery as well as aerosol delivery. This route of delivery showed action for 15 days [2].

**Pulmonary Delivery:** It is mostly used in the case of pulmonary tuberculosis because the drugs can be targeted to the specific site and increased effectiveness because of the presence of the mucosal membrane [2].

**Intravenous Delivery:** The intravenous delivery of the drugs increases the bioavailability of the drug and has an immediate release of the drug. This route of delivery showed action for about 35 days [2].

**Liposomal Delivery:** Liposomes are comprised of vesicles which comprise the hydrophilic and hydrophobic layers. Because of this liposomal-based drug delivery is used in the treatment of tuberculosis. They are used because they have decreased elimination and increased circulation time [16]. They can be administered either intravenously or in the form of aerosols. When liposomal-based drugs were administered in mice twice weekly for 6 weeks, the *Mycobacterium tuberculosis* bacteria was disappeared in mice. The drugs showed effective action below its therapeutic concentration when loaded in liposomes [2].

**Microemulsions and Nanoemulsions:** They are oil in water dispersions ranging in micro or nanoscale [16]. Microemulsions are preferred due to its thermodynamic stability, greater diffusion rates, greater absorption rates, easy to prepare and it has higher solubility. They have increased bio-availability and have decreased side effects. If the prepared formulation is stable, then the size of the drops is in nanometer range so it can also be termed as nanoemulsions [2].

According to the study carried out by Mehta *et al*., tween 80 was used as a surfactant used in the preparation of microemulsion of anti-tuberculous drug rifampicin. Oleic and phosphate buffer were also used in the preparation of the formulation. After conducting many studies, it was concluded that the formulation was stable [17].

**Solid Lipid Nanoparticles:** In this formulation, the drugs are encapsulated in a solid lipid matrix. Both hydrophilic and hydrophobic drugs can be incorporated into the SLN's. They have higher stability and use a reduced amount of organic solvents during the formulation. Only 7 doses of the formulation were enough to cure the disease. The formulation showed activity for 5-10 days [2].

**Niosomes:** Niosomes are thermodynamically stable comprising of non-ionic surfactants and lipid cholesterol in an aqueous medium which may form multilamellar or unilamellar structures. They can be used as carriers for amphiphilic and lipophilic drugs. They are used for controlled and sustained delivery of drugs. The drugs, when loaded in niosomes, shows therapeutic efficacy at lower doses at the targeted site and can also be administered orally [2]. According to the study carried out by Rani *et al*., rifampicin and gatifloxacin were loaded in niosomes for the treatment of tuberculosis.

Two different strains of bacteria were injected. It was concluded that the drug-loaded niosomes showed good therapeutic efficacy, optimum release rate, reduced dose, less number of days for treatment and good patient compliance [18].

**Nanosuspensions:** They are the colloidal dispersions of pure drugs which contain surfactants [19]. It has the homogenous particle size of the drug in the formulation. Many solvents are used to solubilize the drug to get a size range in the nanoscale. It was administered intravenously for mycobacterial infections [16].

**Dendrimers:** They are well-defined macro-molecules which have multiple branches which have compromised functions. They have small molecular weights [19]. The drugs are encapsulated by complexation and conjugation. So, they are used as delivery systems for the treatment of tuberculosis. These show a sustained delivery of the drugs at the targeted site [16].

**Microspheres:** They are discrete particles in which the drugs can be encapsulated or coated onto the surface. They are prepared with polymers to release the drug at the desired time. The duration of drug release from the microspheres can be from days to several months. Usually, lactide-co-glycolide polymers are used due to their biocompatibility. So they can be used as delivery systems in the treatment of tuberculosis [16].

**Oligonucleotides:** Oligonucleotides are used in finding the bacterial strains which are resistant to drugs in the treatment of tuberculosis. They are used in the form of biochips loaded in gel pads. Using the PCR technique different strains of bacteria which are resistant to isoniazid and rifampicin were identified [20].

In the study carried out by Gunter Harth *et al*., he has tested the bacteria which were resistant to anti-tuberculosis drugs by using oligodeoxy-ribonucleotides against mRNA of an enzyme glutamine synthase. This is carried out using antisense ODN technology. Different strains of bacteria were cultured in a medium, and the minimum inhibitory concentration of each was found out. It was found out the antisense oligonucleotides decreased the growth of the bacteria Mycobacterium tuberculosis which led to the decrease in the disease [21].

**Proteins and Peptides:** Anti-tuberculous peptides have advantages such as low immunogenicity and broad mechanism of actions. The anti-tuberculous peptides could be obtained from various sources such as immune cells from humans, non-immune cells from humans, bacteria, fungi, venoms, cyanobacteria, and bacteriophages. They are the synthesized oligopeptides which contain a long chain of amino acids. The human immune cells which respond to anti-tuberculous peptides are cathelicidins, defensins and granulysin. The human non-immune cells which respond to anti-tuberculous peptides are hepcidins and $HCl_2$. The anti-tuberculous peptides obtained from bacteria are nixin, lacticin 3147 and E50-52 oral lassomycin. This process was opted due to the severe side effects of the present anti-tubercular drugs. The author has concluded that the use of peptides in the treatment of tuberculosis can decrease the side effects and increase the therapeutic efficacy [22]. Guomiao Shein *et al*., in his study have found out different antibodies for the obtained antigens which were found in the experimental procedures. BFrB and TrxC antigens were used in the study. They were tested on HIV positive TB patients as well as HIV negative TB patients. It was found that only a few selective peptides have shown results on the sera. It was also mentioned that the peptides used were stable during the study and showed results [23].

**12. Alternative Delivery System for Targeting Tuberculous Meningitis (Nasal Delivery Targeting BBB):** The blood-brain barrier is a complex structure or barrier which is present in the cerebrovascular endothelium and is responsible for protecting the brain from the entry of the toxic substances. The blood-brain barrier is composed of astrocytes, pericytes, and endothelial cells. It is a barrier between the blood and the central nervous system [24]. It restricts the entry of particles into the brain. So, due to the presence of this barrier very few drugs can enter into the brain crossing the BBB. Blood-cerebrospinal fluid barrier is a part of the BBB which is present within the arachnoid membrane. It helps in separating the blood from the CSF [25].

Due to the absence of intercellular clefts, fenestrae, and pinocytosis, there is a decrease in the permeability of substances into the brain. There is a greater difficulty for the hydrophilic molecules to cross the BBB [25]. Hydrophilic substances, peptides, proteins, cationic and anionic molecules are difficult to cross the BBB. It is easy only for the lipophilic substances to cross the BBB [26].

**Numerous Strategies of Drug Delivery to Target the Brain:** There are 3 main strategies to target the brain. They are invasive, non-invasive and recent advancements in brain-targeted drug delivery. The different invasive technologies are chemical disruption of the blood-brain barrier, focus ultrasound-enhanced delivery, craniotomy based drug delivery, convection-enhanced delivery, and polymeric wafers and microchip technology. The different non-invasive technologies are efflux pump inhibition, prodrug approach, cell-based therapy, nanocarriers as drug delivery systems and intranasal drug delivery. The novel technologies in drug delivery to the brain are antibodies mediated drug delivery, Mfsd2a based drug delivery, facial intradermal injection and laser light technology [26].

Nasal route is an important route of drug delivery targeting to the brain. It has rapid systemic circulation as compared to the intravenous delivery [27]. It is a non-invasive method of delivery of drug to the brain and the spinal cord. The nasal route allows the delivery of those drugs which cannot cross the BBB through any other route [28]. The main purpose of this delivery system is that it can enter the brain easily by crossing the BBB. The drug is targeted to the brain through the trigeminal and olfactory region. It is because the olfactory nerves start from the brain and end at the olfactory epithelium in the nasal cavity due to which there is easy delivery of drugs to the brain. The olfactory region comprises of 3 types of cells, *i.e.*, olfactory receptor cells, basal cells, and epithelial cells. Olfactory receptor cells are the neurons present in the brain in the olfactory bulb which are responsible for the transport of drugs from the nasal cavity to the brain [27]. Oil in water nanoemulsion is the most commonly prepared formulation to administer the drug through the intranasal route [29].

The size of the particles should be small to deliver through the nasal route [30]. The factors affecting the nasal delivery of drugs are molecular weight, [31] hydrophilicity, degradation of enzymes and degree of ionization. To avoid these problems nano-delivery system is used wherein the maximum amount of drug can enter the targeted site [30]. The maximum amount of drug can reach the brain through the intranasal route when compared to the intravenous drug delivery system [31]. The various problems associated for the administration of the drug through the intranasal route are a concentration of the drug, dose of the drug to be administered, the volume of drug to be administered, mucociliary clearance and presence of enzymatic activity. These problems can be overcome by prodrug approach, innovative formulation approach, absorption enhancers and enzyme inhibitors and use of different nasal drug delivery devices [32].

Ravi Theaj Prakash U. *et al*., in the study has compared the different routes of drug administration of microemulsions. He has compared oral, different parenteral routes, transdermal and intranasal route of drug delivery. He has reported that there was a maximum amount of drug delivery to the targeted site through the intranasal route when compared to other routes of drug administration [33]. According to Rahisuddin *et al*., intranasal drug delivery is also accepted by the Ayurvedic system of medicine when compared to the oral delivery system due to the decreased dose, decreased side effects and increased therapeutic efficacy and bioavailability of the drug at the targeted site. It is also accepted because the gastrointestinal degradation of the drug administered is also avoided [34].

The treatment of diseases through the intranasal route is termed as nasal karma in Ayurveda [35]. Hydrophobic particles which have a small molecular size can easily cross the BBB whereas there is a difficulty for the hydrophilic substances to cross the BBB. Shashank Soni *et al*., in his study have used the pressurised olfactory drug delivery device to administer the drug through the nasal route. This device administers the drug into the nasal cavity in the form of a spray. This method is not still in use it is in the clinical trials and only after checking the spray volume and therapeutic effectiveness it can be marketed [36].

**13. Novel Advances in MTB Treatment:** BCG vaccine is the vaccine given usually to prevent the occurrence of tuberculosis. But even after the administration of the vaccine, there are many cases of tuberculosis worldwide. So, many different vaccinations are discovered and are in the different stages of clinical trials. The TB vaccines which are in the preclinical phase are Prime-boost vaccines, H64 + CAF01, and rBCGΔais1/zmp1. The vaccines which are in phase 1 clinical trial are MVA85A, MTBVAC, ChAdOx1.85A / MVA85A, Ad5 Ag85A, and TB/ FLU-04L. The vaccines which are in phase 2a of clinical trials are RUTI, H1/H56: IC31, H4: IC31 and ID93 + GLA-SE. The vaccines which are in the phase 2b of clinical trials are VPM 1002 (rBCGΔureC: Hly), M72 + AS01E and DAR-901. The vaccine in phase 3 clinical trials is DAR-901. These vaccines may be injected intravenously, administered orally or intranasally by inhalation. These vaccines are invented to minimize the number of tuberculosis patients in the world which is one of the major causes of mortality [36, 56].

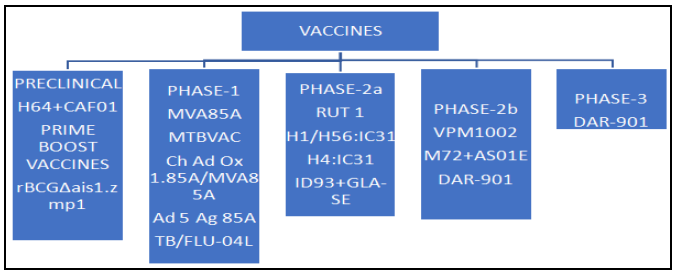


**FIG. 2: DIFFERENT VACCINES WHICH ARE IN DIFFERENT PHASES OF CLINICAL TRIALS**

The new drugs in the treatment of tuberculosis which are in the different phases of clinical trials are:

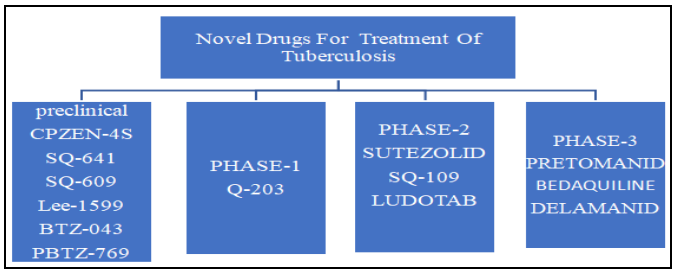


**FIG. 3: NOVEL DRUGS WHICH CAN BE USED TO TREAT TUBERCULOSIS WHICH ARE IN DIFFERENT PHASES OF CLINICAL TRIALS**

The drug sudoterb is used in combination with the current anti-tuberculous drugs to clear the Mycobacterium tuberculosis from the lungs or spleen. Sutezolid has a better bacterial action against *Mycobacterium tuberculosis* and *M. avium* and is effective in the dose of 600 mg twice daily. Pretomanid is usually given in combination with bedaquiline and linezolid [38].

According to Indu Pal Kaur *et al*., it was mentioned that streptomycin is the only antibiotic used in the treatment of tuberculosis. Streptomycin is usually not prescribed usually because it has side effects such as it cannot cross the BBB, leads to nephrotoxicity as it is excreted unchanged in urine and it also causes ototoxicity. Due to these reasons, it should not be administered for more than 2-3 months. So, to minimize the side effects and to target the brain nanoparticle-based intranasal delivery of the drug is preferred. Due to the lipoidal nature of the SLN, the drug is loaded in it as it can be easily absorbed in the brain. It was incorporated to increase drug absorption and transport through the nasal mucosa.

It is also mentioned that to increase the concentration of the drug in the brain and to cross the BBB streptomycin is incorporated into solid lipid nanoparticles. The formulation showed enhanced brain delivery, controlled release and decreased side effects. The amount of drug entering the brain when injected intravenously and when administered intranasally and it showed that intranasal administration showed more penetration into the brain. It can be concluded that the intranasal administration of Streptomycin based SLN had better patient compliance with a lower dose of a drug which can reduce the severe side effects caused by the drug. Due to this, the restricted duration of therapy of 2-3 months can be increased from 9months to one and a half years [39].

In the study conducted by Lisa C du Toit *et al*., it was found that the due to the formulating isoniazid in the form of nanoparticles there would be a decrease in the amount of drug that is to be administered and there would a decrease in the hepatotoxicity. It was formulated in combination with methacrylic acid-ethyl acrylate polymer by which there was a decrease in the dose to once daily. Phase separation and solvent evaporation technique were used in the preparation of nanoparticles. In this study, two methods were followed in the preparation of nanoparticles such as

preparation of nanosystem by emulsion-based salting out approach and preparation of nanosystem by an aqueous based salting out approach. It was found out that the nanoparticles formed by emulsion-based salting out technique had a good construction in size and shape when tested in SEM and Zetasizer studies. In the SEM studies, it was found that the diameter of nanoparticles formed by emulsion-based salting out method was small than the aqueous based salting out approach. In the DoE studies by using Plackett Burman design, it was found that by an increase in the concentration of the polymer lead to the increase in the yield and the aqueous based nanosystem formulation had 100% drug release in a short period [40].

In the study conducted by Rajesh Pandey *et al*., he has used all the 4 first line anti-tubercular drugs by encapsulating it with a PLG copolymer. It was found out that the formulation had a prolonged action when used along with a copolymer. Ethambutol is used in combination because of the inability of streptomycin to be given orally. The method used in the preparation of nanoparticle was a multiple emulsion and solvent evaporation technique. In this technique by zetasizer technique, it was found that the particles were obtained in a nano-size range. It was also found that the $C_{max}$ was less and there was an increase in the $t_{max}$ in the PLG loaded nanoparticles when compared with the free drugs. It was also found that the free drug was present for a short period in the body when compared to the PLG loaded drugs [41].

In another study carried out by Rajesh Pandey *et al*., for inhalable delivery of the anti-tuberculous drugs the drugs were coated with PLG which is a polymer and the nanoparticles were prepared by multiple emulsion techniques and they were then subjected to vacuum and were then subjected to nebulization. The particle size and polydispersity index of the formulation was measured using a Zetasizer. Then the drug was administered using a compressed nebulizer by dissolving the drug in a saline solution. The majority of the nanoparticles were in the nanoparticle range and had a narrow polydispersity index. It was also reported that the maximum amount of nanoparticles were inhaled and reached the site of action. When compared to the oral administration of drugs the drugs loaded with polymer given by nebulization showed action for up to 8 days. The formulation took a long time to attain $C_{max}$ and the AUC values were greater when compared with the normal drug. There was a 28 fold increase in the bioavailability of the polymer loaded formulation when compared with the normal formulation [42].

In the study carried out by Rajesh Pandey *et al*., for subcutaneous delivery of the antitubercular drugs. Even in this study, the drugs were loaded with PLG nanoparticles by multiple emulsion techniques and then the drug-loaded PLG nanoparticles were dried in vacuum. Then the particle size of the nanoparticles was determined by photon-electron microscopy. The drug was injected by mixing the drug in normal saline and was injected by the subcutaneous route. The amount of drug present in the body was found out by collecting the blood samples at different time intervals, and the AUC was plotted. A single subcutaneous dose of PLG loaded drug nanoparticles had a drastic increase in the bioavailability of the drug and the residence time of the drug was increased to 32 days, and the therapeutic concentration was found up to 36 days when compared with the normal drug formulation loaded subcutaneously [43].

From the above-described novel technological studies for the anti-tubercular treatment of the drugs isoniazid, rifampicin, and pyrazinamide, when loaded with a polymer, had an increase in the bioavailability and there was a drastic increase in the retention time of the drug at the site of action. So, the nanoformulation of the drugs with the polymer exerts an increased therapeutic effect and also there is a decrease in the dosing interval as the retention time of the drug at the site of action is increased when compared to the conventional formulation of the drug. To summarise the results reveals there is an increase in the availability of the polymer loaded drug irrespective of the route of drug administration.

**14. Animal Models Used in the Treatment of Tuberculosis:** In the study conducted by Rogelio Hernandez *et al*., used MALB mice in the study for the treatment of tuberculosis. The drug was injected intratracheally for the treatment of meningeal tuberculosis. In this study, the effectiveness of the treatment was compared between the TB bacteria obtained from patients suffering from tuberculous

meningitis. In this technique first, the male BALB mice were infected with different strains of the disease intratracheally. Then the mice were divided into various groups, and the experiment was carried out by examining the mice by euthanizing the mice periodically and also collecting the blood samples at predetermined intervals. Then the histopathological studies were performed in the 3 lung lobes and the other 3 lung lobes were used for immunological analysis. Then the brain of the infected animals was divided into two hemispheres wherein one hemisphere was used for histological studies, and the other hemisphere was used for other studies. The CFU of the lung and the hemispheres obtained from the rat are compared. The bacterial lesions were found after the third and fourth weeks of induction of infection. After 60 to 120 days bigger nodules of infection were seen. Even acid-fast bacilli were present in the brain which did not produce any inflammatory response. Finally, it was concluded that some strains of bacteria which were obtained from the patients caused meningeal tuberculosis in the rats [45].

**TABLE 2: ANIMAL MODELS USED IN THE TREATMENT OF TUBERCULOSIS [44]**

| S. no. | Year | Author | Animal model | Mechanism |
|---|---|---|---|---|
| 1 | 1929 | Shope and Lewis | Guinea pigs | The emulsion of the guinea pigs was infected with sputum of TB patients which caused meningitis and leg paralysis in the animals |
| 2 | 1932 | Burn and Finley | Guinea pigs | Introduced a model of TBM which caused hyper-sensitivity instead of CNS illness. The animals were injected with tuberculosis bacteria and injected with tuberculin at intervals which helped in the development of CNS Tb. |
| 3 | 1933 | Rich and Mc Cordck | Rabbits | Studied the pathophysiology of CNS Tb and the mode of entry of bacteria into the brain. The passage of bacteria through the Rich foci was studied by them |
| 4 | 1933 | Pierce *et al*., | Mice | First person to examine CNS Tb in mice. They were the first to culture the bacteria in liquid culture medium to enable the increased growth of bacteria which led to the formation of many colonies which were injected intracerebrally. |
| 5 | | Lee *et al*., | Mouse model | Studied the formation of granulomas in the brain. It was mentioned regarding the CD4 T-cells which produced interferons and interleukins which led to the activation of cytokine production |
| 6 | | Mazzolla *et al*., | BALB/c and DBA/2 mice | Role of Nramp1 protein in 2 strains of mice was studied. In comparison it was found that BALB/c mice produced infection earlier than DBA/2 mice |
| 7 | | Van Weel *et al*., | 6 weeks old female mice | Mice were infected intracerebrally with the colonies of the mycobacterium formed. Then studies revealed that there was a presence of bacteria in the meninges and perivascular area. The sensitivity of the bacteria was found through CSF studies |
| 8 | 2010 | Hernandez Pando *et al*., | BALB/c mice | The Mycobacterium strain was injected intratracheally. These strains were obtained from the CSF of the patients infected with tuberculous meningitis |
| 9 | 1997 | Bolin *et al*., | Infant pigs | TB was developed in infant pigs, and the bacteria was found in the lesions of lungs, and lymph nodes and subarachnoid granulomas were found in the parenchyma of the brain |

According to the study carried out by John J Roord *et al*., the author has found out an *in-vivo* model for the treatment of tuberculous meningitis in children. The bacteria were injected intracerebrally, and the amount of cytokines and chemokines produced were measured. 6 week old female mice were taken for the study and the bacteria which were cultured in medium containing some amount of tween 80 was injected into the mice intracerebrally. A properly calibrated needle was used to reach the sub-arachnoid space in the cerebral region. Then the mice were euthanized and the brain, spleen, and lungs were removed, and half of the samples were stored in formalin solution and the remaining half portion was stored in sterile 0.9% NaCl solution. Then the procedures were carried out to find out the concentration of cytokines and chemokines produced in the mice organs. It was concluded that

there was a presence of the bacteria in the meninges in the brain and even inflammation was also found in the lungs and spleen of the tested animals. Even the number of colonies formed in the tissue culture studies was also studied. With the histopathological studies also showed that it produced meningitis. When compared with the number of bacteria present in the brain, lungs, and spleen it was found that the meninges of the brain contained more number of bacteria [46].

According to the study carried out by Nicholas A Be *et al*., the study was carried out on the BALB mice for the treatment of tuberculous meningitis. The disease caused in the brain and lungs tissue. Different strains of tuberculosis bacteria were grown in the lab in different methods, *i.e.*, plastic roller bottles or plastic tubes. Only the ones which formed colonies were selected for the study. Then the grown bacteria were injected into the mice intravenously through the tail vein. Then the mice were euthanized using isoflurane in the specified interval of time; then the blood samples were collected from the lungs of the mice. Lungs and brains of the mice were obtained and were stored in the media. The number of colonies of bacteria formed in the parts of the mice was also observed on different days (1, 7, 14 and 28 days). Even the amount of cytokines and chemokines present was also observed by the ELISA technique.

It was concluded that many colonies of bacteria were found in the brain tissue of the mice. The levels of cytokines and chemokines were lower in the brain when compared to the lungs. The presence of different mutants of *Mycobacterium tuberculosis* was also assessed on different days and was found that the mutants were mostly found in the brain tissue than that of the lungs. The data was not collected for several months because the mice were dead by the end of 7-8 weeks after administration of a high dose of intravenous infection. If the mice were to be alive and they were monitored for cytokine levels, it was assumed that it would be higher in the brain. Hence, it was concluded that there is a specific mutant of the bacterium *Mycobacterium tuberculosis* which causes tuberculous meningitis [47].

De Groote *et al*., in the article has compared the effect of the drug on two mouse models with 3 dosage regimens, *i.e.*, intravenous administration, a low dose aerosol, and high dose aerosol model. Isoniazid, pyrazinamide, rifampicin, and moxifloxacin were used in the study for the treatment of tuberculosis. These drugs were used in two combinations, *i.e.*, isoniazid, pyrazinamide and rifampicin and in the second isoniazid was replaced with moxifloxacin. Two strains of mice were used and were infected with the same *M. tuberculosis* strain to produce the disease. For LDA and HDA the bacteria were given through via inhalation using a nebulizer but for intravenous administration, it was injected through a tail vein.

After the therapy, the mice were euthanized and the spleen, lungs, and liver of the mice were separated and were stored in saline solution. The drug was administered orally, and the blood samples were obtained at different intervals and were tested. It was found that the mice infected via nasal route show an early response to treatment when compared to the mice infected intravenously. Isoniazid and moxifloxacin containing combinations showed the same efficacy when administered intravenously. Different dosing of rifampicin to mice did not affect the therapeutic efficacy in mice which were infected with HDA. The results of the mouse studies showed that the killing kinetics of the drug regimens in the lungs were significantly slower for the i.v. infected versus aerosol infected animals in the lungs as well as in spleens [48].

Aliabbas A Hussain *et al*., in this article has developed an animal model for the treatment of CNS Tb by obtaining the samples from the cerebrospinal fluid of the infected people, and it was injected in mice intravenously. The strain of the bacilli used was C3 strain. The cultures were prepared for the growth of the bacteria, and the number of colony forming units was observed.

Female BALB/c mice which were 6-8 weeks old were used for the study. Mice were infected intravenously through the tail vein, and few mice were euthanized at the end of 30 and 50 days to check the number of bacteria in the brain and lungs. The mice infected showed a greater count of bacilli in the lungs in the end of 30 days, and the bacteria were in greater number in the brain in the end of 50 days and no bacteria were found in the control

mice. As the infection produced in the mice was increasing the mortality rate of the mice also increased [49].

Liana Tsenova *et al*., has conducted the study on rabbits for tuberculous meningitis. The disease was injected into animals by using different strains of bacteria *i.e*., CDC1551, HN878 and two members of W/Beijing family. The bacilli were injected into the CSF intra-cisternal after anaesthetizing and immobilizing the animals. The CSF of the animals was obtained every week for 8 weeks. Parts of brain, lungs, liver, and spleen were used for the study of the number of colonies formed, and the remaining parts were stored in formalin acetate for histopathological studies.

HN878 strain of bacilli caused more infection in the brain and cerebrospinal fluid when compared to the other strains of bacilli and developed severe meningitis [50]. The animal models used for the study of tuberculous meningitis since 1929 was compared till the latest models. The injection of bacteria into the animals *via* intravenously, intracerebrally, or intranasally through aerosol. In the studies conducted by researchers on different strains of mice. They have compared the number of bacilli entering the meninges, spleen, and lungs. It reveals that number of bacilli entering the brain when compared to the other organs. So, this led to tuberculous meningitis in the specific animal models. Even in the rabbit model for tuberculosis there found to be many numbers of bacilli entering the meninges of a specific strain of the tuberculosis bacilli when injected intracisternally.

**15. *In-vitro* Cell-Line Models to Cross the BBB:** The passage of substances through the BBB is a tedious task because of the presence of tight junctions and very few fenestrations which allows the entry of very few substances through it. So, due to this reason very few substances can cross the BBB like lipophilic substances of smaller particle size but, the hydrophilic substances cannot pass through it.

So, there is a difficulty in treating the diseases related to the brain as there will be very less amount of drug reaching the site or the drug won't reach the site [52]. So, due to this reason, various cell-lines are used to find out the amount of drug crossing the BBB.

**TABLE 3: DIFFERENT TYPES OF CELL LINES [52, 53]**

| S. no. | Name of cell-line | Type of model | Use |
|---|---|---|---|
| 1 | RBE4 cell-line | Mono-culture model | It is the primary cell-line of the brain used to study the penetration of the drug through BBB and to cure brain disorders |
| 2 | Human microvascular endothelial cell-line | Mono-culture model | It is an endothelial cell lie which is obtained from the endothelial cells of the brain which used to find out penetration of the drug in tuberculous meningitis |
| 3 | Endothelial cells + astrocytes | Co-culture model | It is a cell-line comprising of both endothelial cells and astrocytes. It is mostly used for drug delivery studies. When both these cells are combined, they form more rigid tight junctions which resemble the BBB |
| 4 | Endothelial cells + pericytes | Co-culture model | This mixture is preferred because the pericytes and endothelial cells are more connected in the BBB. It also mostly resembles the structure of BBB |

**CONCLUSION:** Resistance to the first line drugs are becoming more complicated/tedious to the formulation scientists. Researchers are to be emphasized more on the resistance parts hence to create more awareness on the complication of the disease and its treatment. Many researchers suggest that intranasal delivery of the drugs to target the brain to treat the disease. Promising results are reported for the lipid-based drug delivery system for nasal delivery to address the issues of BBB and CSFB and hence the treatment of TBM.

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