DIAGNOSIS AND MANAGEMENT OF TUBERCULOSIS

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Diagnosis & Management of Tuberculosis

Chapter 1

Tuberculosis: Propagation beyond Lungs

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Abstract

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) still remains a formidable global health challenge particularly considering the fact that one-third of the world's population are infected by TB as currently estimated by World Health Organization. The problem is compounded by the fact that roughly 10% of the infected people are symptomatic. Although TB affects the lungs in 80% of cases, however, in the remaining 20% the disease may affect other organs severely if lately diagnosed or left untreated. Hence, it is pertinent not only to diagnose early and initiate appropriate therapy but understand the pathogenesis of TB and its different types to prevent transmission. This chapter provides a consolidated gist of the different types of TB viz. Meningitis TB, Occular TB, Lymph node TB, Spinal TB, Cutaneous TB, Hepatic TB, Renal TB, Abdominal TB and Genital TB at common platform.

Keywords: *Mycobacterium tuberculosis*; pulmonary tuberculosis; extrapulmonary tuberculosis; diagnosis, pathogenesis

1. Introduction

Tuberculosis (TB) is an infection caused by Mycobacterium tuberculosis (MTB) causing global concern. According to estimates, in 2015, approximately 9.6 million people suffered with TB, of which 1.5 million died [1]. Several genetic, social, environmental and biological determinants of health have been instinctively recognized as risk factors for TB. Among them, human immunodeficiency virus (HIV) infection and diabetes have fuelled the resurgence of

TB worldwide.

TB can affect any organ system in the body despite pulmonary TB being the most common presentation. However, extrapulmonary tuberculosis (EPTB) is also an important clinical problem which has compounded the pathogenesis of TB. Types of EPTB that have been widely reported are Meningitis TB, Occular TB, Lymph node TB, Spinal TB, Cutaneous TB, Hepatic TB, Renal TB, Abdominal TB and Genital TB. In this chapter, we made an attempt to give a brief overview of the most prevailing forms of EPTB (Fig. 1). We have tried to give an account of the various types of EPTB and explain in brief the pathogenesis and current treatment methodologies.

2. Pulmonary Tuberculosis

TB or pulmonary tuberculosis (PTB) is caused by the intracellular bacterium MTB. PTB has been a major health concern since antiquity. TB infects around 8 million people every year leading to approximately 3 million deaths every year. These numbers may increase in the coming years due to increasing HIV patients and the emergence of Multi drug resistance (MDR) [2-4].TB can present as an asymptomatic infection to a life-threatening disease. TB infections can be classified as active TB disease, which is transmissible (active TB) or latent TB infection (LTBI), which is an asymptomatic and non-transmissible state. It is estimated that in 2014, around 9.6 million people developed active TB disease, among 1.5 million died [5].

2.1. Epidemiology and risk factors

Developing active TB infections are quite frequent in exposed infants, but much lower in children 2–10 years of age; risk then rises during adolescence and plateaus around 25 years of age and remaining high throughout adult life. Incidence of active TB infections are approximately two fold higher in men than in women, and approximately 10% of all new cases worldwide occur in children [6,7]. HIV infection is the strongest risk factor for TB; 12% of all new active TB disease cases and 25% of all TB-related deaths occur in HIV-positive individuals. For example, majority (75%) of HIV-associated active TB disease cases and deaths occur in Africa (8). Other risk factors are responsible for the remaining fraction of TB cases in the general population. Other risk factors for TB include type 2 diabetes mellitus, alcoholism and smoking. Therefore, addressing these social and behavioral determinants could help to expand the current biomedical paradigm for TB control [8].

2.2. Pathogenesis and clinical features

Pulmonary alveoli are initial site for the infection, where MTB invade and replicate within alveolar macrophages. Then the inhaled mycobacteria are phagocytized by alveolar macrophages, where macrophages interact with T lymphocytes, resulting in differentiation of

macrophages into epithelioid histiocytes [9]. In the granuloma, CD4 T lymphocytes secrete interferon-γ, which activate macrophages to destroy the bacteria. CD8 T lymphocytes also directly kill the infected cells [10]. It deserves special mention that, bacteria are not always eliminated from the granuloma and they can become dormant resulting in a latent infection. Gon focus in the lungs either enlarges as disease progresses or undergoes healing. At early infection process, MTB commonly spread via lymphatic channels to regional hilar and mediastinal lymph nodes via the bloodstream to more distant sites in the body. The initial infection is generally clinically silent. Approximately 5% of infected individuals show inadequate immunity and clinically active disease develops within 1 year of infection. For most infected individuals, however, TB remains clinically and microbiologically latent for many years for most infected individuals [11]. Endogenous reactivation of latent infection develops many years after the initial infection. This reactivation predominantly involve the apical and posterior segments of the upper lobes and the superior segments of the lower lobes most likely due to a combination of higher oxygen tension and impaired lymphatic drainage in these regions [12]. Progressive extension of inflammation and necrosis, with frequent development of communication with the airways and cavity formation are the main abnormalities during the reactivated PTB.

2.3. Diagnosis

Diagnosis of PTB is challenging due to the difficulty in culturing this slow-growing organism in the laboratory. One way is to culture MTB from a specimen taken from the patient. Tuberculin skin test (TST) is the most common method and has been used for years to diagnose the latent TB in non-immunized person. However, TST has limitations such as false positive test results in Bacille Calmette-Guérin (BCG) vaccinated individual and in individuals with non mycobacterial infections [9,10,13,14]. A new interferon-γ assay has been introduced for the diagnosis of LTB infection, which is found to be reliable method than the TST [11,15]. This test is comparatively cheap and fast TB testing. This new test use polymerase chain reaction (PCR) detection of bacterial DNA and whole-blood interferon-γ assay [12,16]. Chest radiography is a more expensive test but important, especially when clinical suspicion of PTB exists but the sputum is still negative. In HIV patients, the radiological appearances are often less specific as symptoms and signs may not appear to be classical and sputum also may be negative on direct smear. The nucleic acid amplification test detects the MTB nucleic acid sequence using an amplification technique [17,13].

2.4. Management

LTBI treatment regimens recommended by the WHO include 6–9 months of isoniazid, 3 months of rifampicin plus isoniazid, 3–4 months of isoniazid plus rifampicin or 3–4 months of rifampicin alone. All regimens are known to be efficacious, but patient compliance can be poor with the longer regimens [18,19]. Rifampicin-containing regimens are shorter and might

be more suitable in populations with a high prevalence of isoniazid mono-resistant strains. It is always important to ensure adherence and provide patients with adequate counseling regardless of the regimen. The current preferred regimen for active TB disease is a minimum of 6 months of therapy with, first line drugs like isoniazid, rifampicin pyrazinamide and ethambutol during the first 2 months (the intensive phase of treatment), followed by isoniazid and rifampicin for 4 months (the continuation phase) [20,21]. Treatment efficacy and progress are usually monitored with repeat sputum smears, cultures and chest X rays. Although the standard 6 month regimen has a high success rate (approximately 86% under routine, program¬matic field conditions; the regimen itself has higher efficacy), it also has several limitations due to long duration of the treatment. The adverse effects range from gastrointestinal intolerance to severe adverse effects such as hepatitis, immune thrombocytopaenia, agranulocytosis, haemolysis, renal failure, optic neuritis and ototoxicity.

It is always necessary to ensure optimal adherence as lack of treatment completion also contributes to treatment failure, relapse and the emergence of drug resistance. Directly observed therapy (DOT) is the most common adherence monitoring approach. However, various alternative methods are now being tried out to improve adherence, including mobile phone reminders, smart pill boxes, video DOT and the use of call centres to follow-up with patients. Beyond drug therapy, there is a role for surgery in the management of drug-resistant TB. In patients with unilateral disease or apical bilateral disease with adequate lung function, surgical treatment to remove the entire affected area of the lung could be effective. However, in patients with resistant TB, elective partial lung resection (lobectomy or wedge resection) is associated with improved treatment success [22,23].

At present BCG is the only currently licensed vaccine to prevent the development of active TB disease [24]. The BCG vaccine was first used in humans in 1921 and has been evaluated in numerous interventional trials and observational studies looking at less common manifestations of active TB disease. The efficacy of the BCG vaccine against pulmonary TB in adults has been reported to be 0–80% [25]. Despite the variability in its efficacy, BCG vaccine has proven that protective immunity against TB can be induced by a vaccine. Indeed, the main goal of current vaccination research is to help prevent active TB infection from developing in the 10% of infected individuals who cannot contain the infection on their own as LTBI. Since, the BCG vaccine provides only limited protection for neonates and children and no protection against pulmonary TB in adults, it accounts for most of the TB cases worldwide.

Current tools and strategies for diagnosis of TB are inadequate, however, notable advances in TB diagnostic technologies have been made in the past several years, and the potential exists for translating these improvements into meaningful developments in global TB clinical care and control. The current first-line treatment for TB is a multidrug regimen consisting of rifampin, isoniazid, pyrazinamide, and ethambutol. Clearly, there is an urgent need

to improve treatment by introducing new drugs. Potential new agents should reduce treatment duration, have an acceptable tolerability, active against MDR/XDR TB, can be used in HIV-infected patients with TB and be active against latent TB Different classes of drugs like Nitroimidazopyrans, Diamines, Fluoroquinolones and Diarylquinolines are currently being investigated in clinical trials [26]. Besides developing new anti-tuberculosis drugs, introducing efficacious vaccine is needed for control and prevention. Numerous attempts for obtaining MTB vaccine with enhanced protection over BCG, durability, and safety have been made. Candidate vaccines against MTB have largely focused on targeting immunodominant antigens that are secreted proteins, including Ag85A , Ag85B, ESAT-6, TB10.4, Rv1196 and Rv0125 [27]. Development of new vaccines and diagnostics will be aided by the discovery of additional antigens relevant to both natural and vaccine-induced immune responses to MTB infection. The development of TB vaccines faces numerous challenges. Despite these limitations, present 13 vaccine candidates are currently being tested clinically, which are classified into three platform types: whole-cell or lysates of mycobacteria, adjuvanted recombinant protein vaccines and viral vector vaccines. The MTB specific antigenic make-up ranges from several thousand antigens in mycobacterial vaccines to fewer in the viral vector and recombinant protein vaccines [28].

3. TB Meningitis

TB Meningitis (TBM) is highly devastating infection that affects central nervous system leading to high rates of death and disability. It is a severe form of TB affecting the meninges (system of membrane enveloping the central nervous system) which is present as sheath surrounding the brain and spine. Central nervous system associated TB manifests primarily as TBM and less commonly as other conditions such as tubercular encephalitis, intracranial tuberculoma, or a tuberculous brain abscess. It is reported that approximately 1% of all cases of active TB and 5-10% of EPTB cases have TBM [29]. It is especially common in children and HIV patients [30].

3.1. Pathogenesis and clinical features

In this, mycobacterium invades into host body by liquid droplets containing bacilli leading to deposition in the lung. The primary infection occurs usually in the lungs and disseminated through the blood stream to the meninges or brain parenchyma which is responsible for development of small subpial or subependymal foci of metastatic caseous lesions called as rich foci. Then the size of rich foci increases until it ruptures into subarachnoid space and cause meningitis [31,32]. In adults the symptoms of TBM involves headache, fever and meningismus (stiff neck) along with focal neurological deficits, alteration in consciousness, generally feeling unwell, irritable, tired and not being able to sleep [33]. Children suffering with meningitis TB normally involve stiff neck, fever, seizures, nausea and vomiting [34,35].

3.2. Diagnosis

The diagnosis of TBM depends upon the detection of MTB bacilli in the cerebrospinal fluid (CSF). The current conventional used method such as cultural technique and microscopy are less sensitive. Therefore various alternative methods such as immunoassay and biochemical tests are used nowadays to diagnose TBM. The staining techniques such as Ziehl- Neelsen, auramine-rhodamine or Kinyoun are applied to detect the acid fast bacilli (AFB) in CSF. In addition high sensitive neuroimaging involving radiological methods such as Magnetic resonance imaging (MRI) and CT scan are available for the diagnosis of TBM. Molecular based techniques including PCR is a new promising method for the detection of MTB DNA in CSF because of its sensitivity, rapidity and specificity [36,37].

4. Ocular TB

TB being a multisystem infectious disease may affect other organs including the eye. The ocular infection occurs in the eye, around the eye or on the surface (intraocular and extraocular). It is estimated that 1.4% of persons with PTB develops ocular manifestations but many patients with ocular TB have no evidence of PTB [38-43]. The most common indicator of ocular TB is uveitis which is usually presented as chronic anterior uveitis, panuveitis or choroiditid. It is reported that 12 patients with intraocular TB, 9 of whom are presented with retinal vasculitis, 2 with choroidal tubercles and 1 with chronic anterior uveitis [44]. Although there exists an association between miliary TB and ocular TB but ocular involvement may not be always associated with HIV positivity [45]. Ocular TB (OTB) leads to decreased visual acuity and other ocular symptoms. The general symptoms of OTB include blurred vision and light sensitivity, Headache and redness of the eye and Inflammation on the infected area of eye.

4.1. Pathogenesis and clinical features

The OTB can infect the eye through several different mechanisms. The most common ocular infection is often the result of hematogenous spread. The uveal tract, retina and optic nerve (i.e. the iris, ciliary body, and choroid) are the coats of the eye most commonly involved because of its high vascular content. Primary ocular infection of the eye is the one in which bacilli enters the body through lids or conjunctiva [46] most frequently infecting the children. Other external tissues which are less infected include cornea, sclera lession, eyelid and conjunctival. Secondary ocular infection may occur by direct action from surrounding tissues or by contamination with the patient's own sputum. Additionally, some other forms of eye infection can occur due to the hypersensitivity reaction, such as phlyctenulosis, retinal vasculitis, and interstitial keratitis.

Inappropriate implementation of the Revised National Tuberculosis Control Programme

(RNTCP) causes precipitation of MDR-TB cases in the community. In this situation, India is not well equipped to prevent the propagation and dissemination of MDR-TB cases. So a new reemerging threat is slowly growing within the Indian population that may arise as a big challenge in future. MDR-TB is a man-made phenomenon poor treatment, poor drugs and poor adherence lead to the development of MDR-TB [11].

4.2. Diagnosis

Microbiological and direct histopathological examination can provide evidence of OTB infection. The physical examination includes sputum smear and culture, PPD test, and chest radiography. Sixty percent of patients with EPTB have no evidence of pulmonary disease, and chest X-rays are normal in cases of latent TB [47-49]. The Interferon-gamma release assay (IGRA) is recommended by the US Food and Drug Administration and other countries. IGRAs such as T-SPOT and QFT are more specific and sensitive than TST in detecting active pulmonary TB infections [50]. T-SPOT is more specific for diagnosing TB that involve uveitis, and serves as a better diagnostic tool if used in conjunction with the TST [51]. Molecular techniques are also used for detection of mycobacterial DNA through PCR. Detection of antibodies against purified cord factor, the most abundant cell wall and antigenic component of MTB, can provide strong evidence of the infection [52]. PCR yields results much faster than mycobacterial cultures, which can require several weeks for a positive result.

5. Lymph node Tuberculosis

Lymph node Tuberculosis (LNTB) or scrofula occurs when MTB infects the lymph nodes. LNTB has been called as the “king’s evil” in the ancient times [53]. LNTB remains the most common form of EPTB in India and other developing countries [54].

5.1. Pathogenecity and clinical features

MTB enters the body and undergoes lymphatic dissemination. Usually, tonsils are the most common routes of entry for the pathogen. At the initial stage of infection, the lymph nodes are discrete. Afterwards, the lymph nodes coalesce and break open due to pus development. Wounds so developed may not heal even for years [53]. Patients with LNTB infections may have symptoms like fever, weight loss, night sweats and fatigue. Distressing cough may be a prominent symptom in LNTB [55].

5.2. Diagnosis

TST is positive in majority of patients of LNTB, the probability of false negative test is less than 10% [56].Thus, TST seems to support the diagnosis and a negative test substantially reduces the likelihood of LNTB. Ultrasound assessment of abdomen and CT scan of the chest may be required in a few patients. Engorged lymph nodes may show hypodense areas with

rim enhancement or calcification. Excision biopsy is done to diagnose LNTB but fine needle aspiration cytology (FNAC) seems to have established itself as a safe, cheap and reliable procedure [57]. Typically, tuberculous lymph nodes show multinucleated giant cells, caseation necrosis and epithelioid cell granulomas. Caseating granulomas are seen in nearly all the biopsy specimens and 77% of the FNAC's [58]. New diagnostic methods such as PCR tests of the tissue to identify MTB became promising recently.

6. Spinal Tuberculosis

Spinal TB (STB) is the most frequent form of skeletal TB and accounts for 50% of all cases of skeletal TB [59]. Most cases of STB are seen primarily in immigrants from endemic countries. STB is one of the oldest diseases and has been found in Egyptian mummies dating back to 3400 BC [60]. The exact incidence and prevalence of STB is not known. Approximately 10% of patients with EPTB have possessed skeletal involvement where spine is the primary site of infection, followed by the hip and knee [59].

6.1 Pathogenesis and clinical features

Spinal involvement is usually a result of hematogenous spread of MTB into the dense vasculature of cancellous bone of the vertebral bodies. Either a pulmonary lesion or infections of the genitourinary system are the primary infection sites. The progression of STB is slow and the illness varies from few months to few years, with average disease duration ranging from four to eleven months. Generally, patients seek advice only when there is severe pain, marked deformity, or neurological symptoms. The clinical features of STB include local pain, tenderness, spasm and stiffness of the muscles, gibbus, a cold abscess, and a prominent spinal deformity. Back pain is the most common symptom of SP. The intensity of pain varies from constant mild aching to severe disabling. Pain is normally localized to the site of involvement and is most common in the thoracic region. Pain may be aggravated by spinal motion, weight bearing and coughing because of advanced disk disruption and spinal instability, nerve root compression and or pathological fracture [61]. Spinal distortion is a characteristic feature of STB. Type of spinal deformity depends on the location of the tuberculous vertebral lesion. Kyphosis type of spinal distortion is the most common spinal distortion which occurs with lesions involving thoracic vertebrae [59]. The severity of this distortion depends on the number of vertebrae involved. An increase in kyphotic deformity may be seen even after the treatment.

6.2. Diagnosis

Biopsy is a valuable method to diagnose of STB infection. DNA amplification techniques such as PCR may facilitate rapid and accurate diagnosis of the disease. Culturing the organisms is slow and may be inaccurate. However, it is still a valuable diagnostic method in order to recognize the causative germs. CT provides bony detail, while MRI evaluates the involvement of

soft tissue and abscess formation. Significant bone destruction can be detected on plain radiographs or CT scan. Imaging studies such as Ultra sound (USG), plain radiography, CT scan and MRI are valuable tools for the diagnosis and accurate evaluation of STB. USG is useful to evaluate of soft tissue masses, joint effusions, abscesses, joint effusions, and involvement of tendon sheath. CT scan is helpful for the detection of joint involvement, presence or absence of periosteal reaction and soft tissue calcifications, sclerosis, and soft tissue abscesses. MRI is the ideal technique to reveal bone marrow changes in tuberculous osteomyelitis and arthritis, joint effusion, and cartilage destruction early and more accurately [62].

7. Cutaneous Tuberculosis

Cutaneous tuberculosis (CTB) is uncommon and not a well defined disease, comprising only 1-1.5% of all extra-pulmonary manifestations. CTB is prevalent among children and women mostly young adults. Recently, a rise in the number of CTB cases has been seen due to the prevalence of MDR strains of MTB [63]. Scrofuloderma, lupus vulgaris and TB verrucosa cutis are the two common CTB forms. CTB is more prevalent in HIV positive patients. Two ways of infection have been seen in CTB. In the primary infection, mycobacterium may enter the body through a contaminated injection. In another way, pathogenesis is seen during the incubation period in those people who have contacted primary infection. During the initial stages of the infection, bacteria enter the bloodstream via the lymph nodes and thoracic duct and hence cause infection.

7.1. Pathogenesis and clinical features

The pathogenecity of CTB depends on the root of infection and the level of cell mediated infection (CMI) of the host [64]. As CMI may deteriorate as a result of illness, immunosuppression, ageing, HIV infection and malnutrition, there is a long-term risk of reactivation. This risk is maximum during two to three years after primary infection. CTB can occur following any injury. During this early stage of the infection, a number of mycobacteria reach the bloodstream [65]. This hematogenous TB takes place for a very short time and is unlikely to continue after delayed hypersensitivity develops. Fever is the main clinical manifestation during this period and lasts for few days. The mycobacteria are seeded at various organs may heal completely or become active again during periods of lowered immunity. Microhematoma that occurs at the injection site PTB patients acts as an area of lowered resistance resulting in seedling of mycobacteria that get fixed at these sites and later progress to abscess formation [66]. It is hypothesized that the high lactic acid content and lymphatic tissue with very rich blood supply and absence of reticuloendothelial cells may help in localization of MTB in the muscles [67]. However, such an occurrence in skin is not yet defined. Patra et al. reported that, BCG vaccination scar was found in 59.62% of cases of TB which reflects the incapability of the vaccine to protect TB completely [68]. Thus, CTB infections should be considered in the

differential diagnosis of any chronic infection or local abscess that forms especially if there has been an interval of 2 to3 weeks between injury and the development of abscess.

7.2. Diagnosis

The only absolute criteria in confirming a diagnosis of CTB is a positive culture of MTB from the biopsy material on Lowenstein Jensen's media (LJ media). AFB smear is useful if lesions have a high bacterial load as seen in case of miliary TB, Lichen Scrofulosorum (LS) and TB gumma. PCR technique is found to be efficient in case of multibacillary forms of CTB. This technique is found to be 50-70% accurate in detecting CTB. However, couple of diagnostic tests is generally performed to precisely confirm the diagnosis. Commonly used tests include testing of blood, urine and sputum samples along with CT scan and X ray of chest and bones. Furthermore, the diagnosis relies on histopathology, PCR and culture on LJ medium. Mantoux test is also conducted during the course of diagnosis [68,69].

8. Hepatic Tuberculosis

Hepatic tuberculosis (HTB) is a common manifestation where TB involvement of liver is seen in up to 50-80% cases. With the increasing risk of TB, the rate of hepatic TB has also been increasing particularly in Asian countries such as Philiipines [70]. Hepatic connection was found clinically in 50 80% of all patients dying of pulmonary TB and in up to 91% on autopsy [71]. It is more common in male as compare to female with the ratio of 2:1. There is no specific age but according to one study more patient of HTB fall within the age range of 11-50 year [72]. HTB can be classified into the following: 1) Localized tuberculous involvement of liver in the form of the primary tuberculous complex with caseation of the associated hepatic hilar lymph nodes. These lymph nodes may develop the source of spread causing early systemic generalization. 2) Miliary TB, a part of wide infection, on the liver by clustered miliary tubercles. 3) Tuberculomas, or granulomatous disease, can occur through enlargement of tubercles foci as well as nodular development of tuberculous foci in the tertiary stage [71].

8.1. Pathogenesis and clinical features

HTB infections can occur prenatally, perinatally and postnatally. Perinatally and Prenatally HTB infections are carried through the umbilical vein or the amniotic fluid and presence of maternal placenta tuberculosa is a pre environment for both infections. Postnatally, tubercle bacilli reach the liver by the way hematogenous dissemination or hepatopetal lymph vessels. In case of millary tubercle bacilli reach the hepatobilary tract through the hepatic artery from a primary tuberculous infection of the lungs. If tubercle bacilli reaches the liver by lymphatic spread or due to break of a tuberculous lymph node in the portal tract, this case includes localized hepatic tuberculous. In some case the tuberculous infection reach the liver naturally through the involvement of gastro intestinal tract [71]. Hematogenous dissemination is more

frequent than lymphatic vessels. In miliary TB, through hematogenous spread, it produces a number of small tuberculous of liver foci. Irrespective of the mode of entry, the liver responds by granuloma formation both in case of caseating and non caseating granulomas. The common sign of HTB includes fever, poor appetite, fatigue, pain in the hepatic region and hepatomegaly. It is often hot in the afternoon with chills and night sweat sometimes. Hepatomegaly is the main sign with more than half of patients having haphalgesia. Mild jaundice can develop in upto 15% patients because of the oppression of nodules against the hepatic ducts and bile ducts [73].

8.2. Diagnosis

It may be difficult to be diagnosed clinically because of lack of specific clinical symptoms, and may be unsuspected or confused with primary or metastatic carcinoma of the liver. The suspected cases need diagnostic examination based on the histological and bacteriological studies, as well as PCR analysis. Approximately 75% of patients with HTB were found to have abnormal chest X rays demonstrating PTB [74]. CT and MRI are used to diagnose tuberculoma or tuberculous liver abscess. HTB could also be confirmed by liver biopsy through diagnostic tool laparoscopy, exploratory laparotomy and finally autopsy [73].

9. Renal Tuberculosis

Renal TB (RTB) is subpart of genitourinary tuberculosis (GUTB). GUTB is the second most common form of EPTB after lymph node involvement [75]. The primary infection through different mechanisms that include direct infection of the kidney and lower urinary tract (renal pelvis, ureter, bladder and urethra). In worldwide 15% of patient infected with HIV in which 75% of patients infected with GUTB as well as co infected with HIV [76].

9.1. Pathogenesis and clinical features

RTB cases are mostly affected by milary tuberculous infection, where milliary lesions found in renal tissue as a consequence of hematogenic dissemination, particularly in the cortical region [77]. Furthermore, in the kidneys, the medullary region is the place for colonization by MTB, where granulomatous lesions form which lead to tissue destruction. The renal lesion starts at the cortex which tends to migrate to the cortico-medullary junction and develop cortical granulomas. These granulomas invade the renal medulla and causes papillitis. With the progression of disease cause papillary necrosis develop cavities that crash the renal parenchyma and can migrate into other collecting system [78]. In clinical presentation typically granulomata lesions start from the kidney and disseminate to the ureters, bladder, and testicles. Early renal disease may present as the proteinuria, pyuria, and loss of kidney function. Hematuria is another possible symptom of renal TB. It may also involve flank, back pain and constitutional symtoms such as fever, weight loss and fatigue [79-81].

9.2. Diagnosis

Diagnosis usually involves isolation of pathogen in urine sample or by tissue biopsy. To evaluate RTB, partially three different urine samples should be collected for acid-fast staining and mycobacterial cultures. There are some specific features in a urine examination that suggest a diagnosis of RTB, such as acid leukocyturia or hematuria and pH, associated with negative urine culture for the usual bacteria that causes urinary tract infection [78]. Some other molecular biology technique is used to diagnosis such as PCR. CT scan is also helpful in determining the extent of renal and extrarenal spread of disease [82].

10. Abdominal Tuberculosis

Abdominal tuberculosis (ATB) is the sixth most common extrapulomary site of TB after lymphatic, genitourinary, bone and joints, military and TBM [83]. The ATB is mostly seen between the age of 25-45 year. It is estimated that approximately 15%-25% of cases with ATB have associated PTB [84,85]. ATB can involve entire gastrointestinal tract, peritoneum, lymph nodes or solid visceral. In ATB, ileocaecal region is the most common site but rarely occur in ascending colon, jejunum, appendix, duodenum, stomach, oesophagus, sigmoid colon and rectum [86]. Infection in this region leads to the formation of granuloma, caseation, fibrosis, scarring, fibrosis and mucosal ulceration. It is usually difficult to diagnose due to its nonspecific clinical presentation, lack of sensitive tool and variable anatomical location.

10.1. Pathogenesis and clinical features

In the ATB, MTB reaches different site of abdomen such as kidney, lymph nodes and peritoneum usually due to ingestion of infected sputum or from infected source such as milk products, haematogenous or lymphatic dissemination of MTB from the pulmonary site. There are different sites involved in ATB viz. gastrointestinal tract, peritoneum, and visceral [87]. GastroIntestinal tuberculosis is one of the most common forms of TB in the developing world that represents 70-78% cases of abdominal TB. The primary site of TB is the lungs but the tubercle bacteria can affect to other parts such as esophagus, small bowel, ileum, duodenum, jejunum due to ingestion of infected sputum or food infected with Mycobacterium bovis resulting into primary intestinal TB. It mostly affect the iieocecal region bur rarely involve esophagus stomach and duodenum [88]. Peritoneal tuberculosis is another form of TB mostly seen in association with gastrointestinal TB that affect 4-10% patient of EPTB [89]. In this case MTB reach to peritoneum usually due to ingestion of infected sputum or by haematogenous spread from military TB or active pulmonary TB. Moreover it can also spread through ruptured lymph nodes or intra-abdominal organ. It occur in wet ascetic type (commonest, approximately 90%), fibrotic fixed type and dry plastic type [90]. Visceral tuberculosis is less common form of TB that affects 15-20% of all abdominal TB patients. It is disseminated through blood from pulmonary site which mainly affects genitourinary organs followed by

liver, spleen and pancreas but the symptoms of visceral TB are non specific which makes difficulty in diagnosis [91]. The symptom of ATB involves weight loss, fever, diarrhea, constipation, fatigue, malaise, abdominal pain, and abdominal distension. Esophagal TB involves dysphagia, retrosternal pain and odynophagia. Gastric outlet obstruction, epigastric pain and an acute episode of vomiting and dyspepsia are seen in duodenal TB. Ileocecal TB may present abdominal pain, malabsorption, nausea and vomiting. In the colonic TB the symptoms may be focal or multifocal with pain, fever, anorexia, weight loss, and change in bowel habits. Rectal and anal TB may present constipation and multiple fistulae symptoms [87-92].

10.2. Diagnosis

The acid fast staining is the current conventional method for diagnosis of PTB but not applicable for diagnosis of various types of ATB. Therefore some alternative radiology techniques are used to diagnose this disease such as radiographic imaging, chest radiograph, CT, and/or USG of the abdomen. CT of the abdomen is useful to investigate the thickened peritoneum, ascites, mesenteric disease, caseation within lymph nodes, bowel wall thickening, bowel obstruction and omental thickening [93-95]. The diagnosis of gastro intestinal is based on endoscopy and colonoscopy. Endocopy shows the intestinal lesion which shows in the form of ulcers, ulcerohypertrophic or hypertropic. Colonoscopic is helpful to find ulcers, nodules, a deformed ileocecal valve and strictures [96-97]. Barium based studies are advanced sensitive method which is useful to diagnose the area of narrowing and ulceration of intestinal TB whereas USG is helpful to diagnose extraintestinal TB [98].

11. Genital Tuberculosis

Genital TB (GTB) is another common form of EPTB, causing morbidity in developing countries [99]. In case of females, there are involvement of different genital organ such as fallopian tubes (90%-100%), endometrium (50–60%), ovaries (20–30%), cervix (5–15%), and rarely vulva and vagina (1%) [100]. It is the major factor of infertility particularly among women. Similarly in case of males, GTB is associated with TB of the kidney, prostate, epididymis, vas deferens, seminal vesicle and testis as well as scrotum may occasionally be affected. It is more common in males [101].

11.1. Pathogenesis and clinical features

The tuberclli bacilli reaches to the genital tract by three routes such as haematogenous route (90%), abdominal visceral such as the bladder, rectum, appendix and intestines or by lymphatic spread. Fallopian tubes are the main source of infection in genital TB. Tuberclli bacilli reach to fallopian tubes by haematogenous then gradually spread to the endometrium [101,103]. Sometimes it can also be spread during sexual contact with infected person. In

spread to seminal vesicle, deferent duct and epididymis but less common is testicular site [102]. GTB presents various clinical symptoms including oligomenorrhoea, amenorrhoea, menorrhagia, abdominal and pelvic pain, dyspareunia, dysmenorrhoea, infertility and some may be asymptomatic [104]. In case of fallopian TB the fallopian tube is normal in the initial stage of infection but some changes appear like mimicking salpingitis isthmica nodosa, nodular transformation in the latter stages. In endometrium TB, hemorrhage, caseous necrosis and ulceration can been seen whereas adhesion with the fimbria or formation of unilateral or bilateral adnexal mass can be seen in ovary. Male patients with GTB clinically present with lower abdominal pain, epididymitis, prostatitis, testicular swelling, discharging scrotal sinus etc [105].

11.2. Diagnosis

The diagnosis of GTB is difficult as the signs and symptoms are nonspecific [106]. Monteux test is common method to diagnosis of GTB especially in women of childbearing age [107]. AFB is more sensitive method to suspect TB which is based on microscopy. For microscopic examination of AFB at least 10000 organisms per ml should be present in sample but the culturing method is more sensitive only requiring 100 organisms per ml and it can take long time to grow on Lwenstein-Jensen (LJ) media near about 8-6 week. Culturing of menstrual fluid obtained from vagina is the best method in women facing the problem of infertility or abnormal bleeding during the GTB. The AFB in vagina, endometrial, cervix and vulva may be diagnosed directly by biopsy method. Laproscopy is used to determine the lesion in the tubes, ovaries and adnexae. In addition, Heteroscopy is also used for visualization of the uterine cavity in GTB [106]. In case of males, histopathological studies of biopsy specimens possibly to diagnose of genitourinary tract TB. AFB or PCR test is useful to detect AFB in genitourinary tract. TB bacilli detect by microscopically and culturing method in prostatic secretion and ejaculation. Auramine staining and genomic amplification can be performed for scrotal purulent. USG is the traditionally method to diagnose the epididymo-orchitis TB [108].

12. Current anti-TB treatment

Anti-TB drugs still remain the stronghold for the treatment of both PTB and EPTB. Recent guidelines recommend the same regimen for both EPTB and PTB. However, the data for the recommendation for most other forms of EPTB is not based on studies as robust as those for PTB. An important obstacle during the TBM for instance is the ability of the blood-brain barrier to limit intracerebral concentrations of anti-TB drugs. While pyrazinamide, isoniazid, protionamide, and cycloserine penetrate well into CSF, p-aminosalicylic acid and ethambutol have poor or no penetration ability. Steptomycin, Rifampicin and kanamycin penetrate the CSF well only in the presence of meningeal inflammation. Fluoroquinolones such as levo-

floxacin and moxifloxacin have variable CSF penetration, with excellent penetration seen in later generation drugs. In a recent phase 2 clinical trial, high-dose intravenous rifampicin with the addition of moxifloxacin led to three times increase in the plasma and CSF area under the concentration time curve and was connected with a survival benefit in TBM patients [109]. Corticosteroids often have been used as an adjunctive in the treatment of EPTB. The available evidence indicates meaningful clinical benefits only in TBM patients. In TBM patients, recent randomized controlled trials and meta-analysis revealed that corticosteroids significantly decrease the mortality. Thus, adjunctive corticosteroids (either prednisolone or dexamethasone) are recommended to all patients, regardless of disease severity [110]. There are inadequate data to recommend adjunctive corticosteroid therapy in the treatment of GUTB. In these cases, the use of corticosteriods does not significantly reduce the development fibrotic complications like intestinal obstruction or ureteric stenosis.

13. Conclusion

TB is still a major challenge among infectious diseases even in the twenty first century. There is an urgent need to understand the pathogenesis of all the different types of TB and develop novel diagnostic and treatment strategies for the efficient therapeutics against of all forms of TB. Furthermore, stigma associated with TB is another major barrier to health care access and impacts the quality of life for individuals affected by TB hence needs to be addressed to avoid health inequalities. At present, despite the disease being completely curable, it is still facing problems regarding failure and relapse & reoccurrence of the disease. In any of these situations, it must be considered a real possibility that the person has drug resistant TB. Further, finding new targets to combat MDR-TB is another important challenge. Over the past two decades, intensive efforts have been made to develop new vaccines for TB that will boost the immune responses that provide improved protection against MTB infections. Organizations such as WHO and Bill & Melinda Gates foundation aim to advance not only universal access to TB prevention, care and control but also guide the global response to threats and promote innovation in the developing countries.

14. Figure

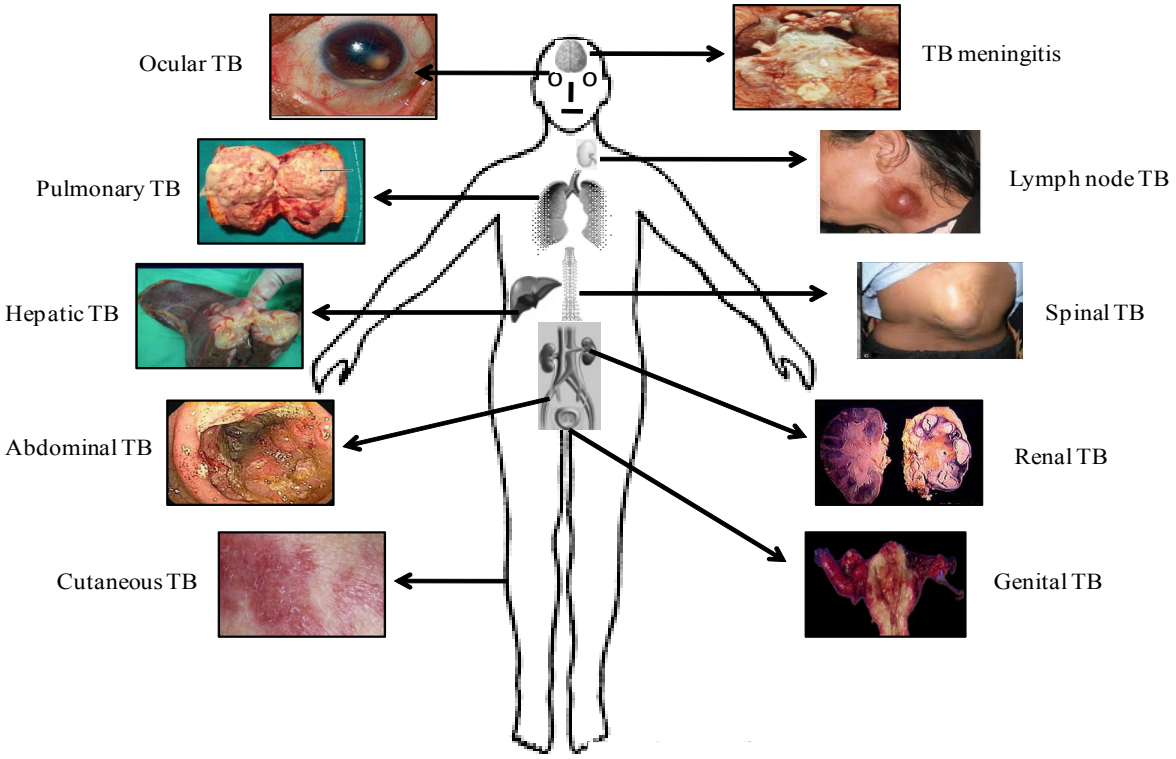

Figure 1. Different types of TB affecting the various anatomical sites of human body (adapted from web resources).

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Diagnosis & Management of Tuberculosis

Chapter 2

Tuberculosis of Rib

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1. Introduction

Even in current era, incidence of tuberculosis remains high in certain parts of the world with more than half of the world's tuberculosis infected population residing in Asia [1]. In countries where tuberculosis is endemic or rampant due to poor socioeconomic status, malnutrition, emergence of multidrug resistant cases (MDR) and increasing association of TB with HIV infection; tuberculosis persists to pose a significant public health problem [2,3]. African population, the inhabitants of the Indian subcontinent and the Caribbean are most frequently affected by this disease.

Musculoskeletal tuberculosis is the most prevalent form of extrapulmonary tuberculosis and it accounts for 10-15% of all the different types of tuberculosis in the developing world; where as in the western world, it constitutes approximately 1-2% of all the cases diagnosed with tuberculosis infection [4]. The common sites affected in skeletal tuberculosis are vertebral column (50%), hip (15%) and the knees (5%). Although any bone in the body can be involved by the tubercular infection, involvement of the ribs is a relatively rare condition and it constitutes approximately 2% of the total cases of musculoskeletal tuberculosis [5,6]. In coming years incidence of this condition is expected to rise; mainly due to emergence of multi-drug-resistant (MDR) strains and due to the ever increasing numbers of immuno-compromised individuals in the given population [7].

2. Pathology

The rarity of the tuberculosis of ribs may be ascribed to its surreptitious beginning and to the infrequency of the involvement of other organs; less than 50% patients have concomitant active pulmonary tuberculosis [8]. However, despite the rarity, tuberculosis remains the commonest inflammatory condition affecting the ribs and it is also implicated as the second commonest cause for rib destruction after metastatic affection [9]. Patients between 15-30 years of age are most often affected by tuberculosis of ribs and mostly males are liable to get diseased as compared to females with this condition.

Although skeletal tuberculosis primarily results from either the lymphatic or hematogenous dissemination of bacilii from the primary infective foci [10,11], but in cases of rib tuberculosis three possible mechanisms have been suggested to describe its pathogenesis [12].

1. Direct extension from the underlying pleural or pulmonary parenchymal disease.

2. Hematogenous dissemination associated with activation of a dormant tuberculous focus.

3. Direct extension from a lymphadenitis in the chest wall.

Direct extension from the underlying pleural or parenchymal disease seems to be a less common cause of rib tuberculosis as compared to the later two [11]. Mostly they present as either osteochondritis at the costochondral junction with or without rib destruction or tuberculous osteomyelitis involving the body of the affected ribs [13]. In some cases, especially in the patients of early ages, it can only involve the anterior chondral part of the rib and even these lesions can subsequently destroy the adjacent bone either due to pressure necrosis or direct involvement of the bone. In less than 10% of cases it may present as a cold abscess over the chest wall [14,15]. These cold abscesses appear at the site of infected lymphnodes that have been invaded by tubercle bacilli [12]. These lymphnodes then become caseous and this caseous material subsequently burrows externally to form a cold abscess over the chest wall [12] Almost in 25% of cases they present as chronic discharging sinuses in the chest wall (Fig.1).

Although we have not come across with post BCG tuberculosis of the rib but rare association with BCG has been postulated. It is not clear that rib tuberculosis has resulted due to direct inoculation of BCG. Many hypothesis have been suggested and post BCG rib TB postulation is stated to be acceptable only after exclusion of all possible modes that could lead to its infection, like: Reactivation of dormant focus, cold suppuration of draining lymph nodes (axillary, internal mammary and supraclavicular), haematogenous spread direct from the attenuated BCG bacilli especially in immunologically suppressed individuals, breaching the first lymph node barrier and spilling into systemic circulation to cause haematogenous spread to reach the rib, etc. Rib affection after BCG has been associated to be with but not as a result of direct inoculation [16,17,18].

3. Clinical Features and Diagnosis

This rare form of extrapulmonary tuberculosis is usually under-diagnosed in many settings. Perhaps the most important cause for under-diagnosis remains the failure of the busy clinicians to think of it; owing to rarity of this condition. Beside tuberculosis there are other conditions, which may mimic this infection clinically and these need to be included in the differential diagnosis of this condition viz. chronic non-specific osteomyelitis of ribs, rib affection following empyema necessitatis, eosionophilic granuloma, syphilis and benign & malignant tumors of rib.

The presenting symptoms of rib tuberculosis can be a swelling in the chest wall, non-healing sinus, cold abscesses and recurrent chest pain with or without any pleura-pulmonary involvement. Commonly they present as painless chest wall mass having variable consistency and size. Local tenderness, with or without erythema of these lesions, usually denotes super-infection of the cold abscess. Mostly they present as solitary lesion and most frequently they involve the shaft of the rib [12]. To establish a diagnosis of this condition may be a difficult task despite the visible lesion on the affected chest wall. In addition to a good history and meticulous clinical examination, use of certain radiological investigations i.e. CXR, CT scan and dedicated assistance and effort from an experienced cytologist for accurate percutaneous needle aspiration for obtaining material for smear, culture and cytopathological examination are essential. Of course, a trucut biopsy, ultrasound guided core biopsy are advocated for obtaining tissue for histopathological confirmation.

These lesions may become apparent on routine chest X-ray (Fig.2); however this imaging modality cannot detect the changes occurring in the early stages of this disease. Therefore, CT scan seems to be the preferred imaging modality to diagnose rib tuberculosis in doubtful cases (Fig.3). Additionally, the CT scan gives a detailed evaluation of chest wall lesion and the intra-thoracic organs, it may also show the nature and extent of the soft tissue lesion, associated intrathoracic lymphadenopathy and bony destruction or erosion [19]. Radiologically, presence of an osteolytic lesion, widening of the rib with added periosteal reaction and the presence of sequestrum - is highly suggestive of a tuberculosis osteomyelitic lesion [11]. There has been significant advancement in the interpretation of CT images. In an invention claimed by Djankaeva OV et al, they utilised the appearances of tissues before and after contrast enhancement, at the area of interest to distinguish among the granulation tissue, capsule of the abscess cavity and the normal tissues [20]. This investigation seems to be useful to know the exact extent of the diseased segment and thereby appears to be helpful in planning surgical intervention or image guided biopsies. Other imaging modalities i.e. Bone scan, MRI and USG may also provide some additional information, which can be helpful in corroborating evidence towards diagnosing these lesions.

Undoubtedly, establishing the presence of acid fast bacilli in the aspirate or tissue from the affected site is confirmatory for diagnosis. Therefore, needle aspiration for smear, culture and cytopathological analysis or histopathological tissue biopsy from the lesion is an essential requirement to reach to a final diagnosis. This biopsy, in addition, is beneficial and helpful to differentiate the diagnosis of rib tuberculosis from other pathological conditions of rib [21]. Understandably, it is imperative and important on the part of treating clinicians to have a pathological or microbiological confirmation of tuberculosis before embarking upon any therapeutic decisions [21]. Currently, the polymerase chain reaction (PCR) is a great contribution; as it allows an early diagnosis, especially when classical bacteriological methods to establish a diagnosis fail.

With the help of the mentioned procedures, a diagnosis of tuberculosis is established through the presence of caseating granuloma with giant cells, acid-fast bacilli in a direct smear or Mycobacterium tuberculosis in culture or in PCR. In clinical practice diagnostic yield of needle aspiration is low ranging from 29% to 36% [12,21]. Therefore if the diagnosis of tuberculosis remains uncertain after needle aspiration, surgical excision of the involved segment for diagnosis becomes mandatory. This surgical procedure for diagnosis must be performed with a therapeutic intent i.e. whole of the affected tissue should be preferably removed leaving the grossly healthy looking cut rib edges. In such cases these surgical interventions minimize the morbidity of the slow to heal wounds and sinuses of the disease of the ribs and surrounding chest wall [22] which the patients have already endured for a long time. Moreover, following surgery, the fibrosis resulting from the long drawn disease affliction is removed and so in the post-operative period, it also promotes neovascularisation in the fresh healing tissue which enhances and aids in better distribution of anti-tubercular medications, thereby increasing the efficacy and the ultimate response to therapy [22]. We recommend that skin should be closed primarily as this will prevent the formation of chronic draining sinus, [12,22] and thus reducing the resulting prolonged morbidity of the patient.

4. Treatment

Based on previous reports [12,21,23], we recommend that if the diagnosis of tuberculosis becomes evident after needle aspiration; the patient should be put on anti-tubercular medication and the diseased area allowed to heal. In doubtful cases or when these lesions do not show any significant improvement despite 1 to 3 months of anti-tubercular medication, then surgical excision must be performed. In endemic areas, many a time, anti tubercular treatment is initiated on presumption and the nature of response then becomes the only clue to the etiology of the disease [12]. Considering the logistic constrains in the countries where tuberculosis is endemic or rampant, commencement of this empirical therapy on clinical grounds cannot be condemned entirely. However one should adopt such an approach with caution; it should be utilised only in highly suspicious cases and it must be discontinued in the absence of prompt

and adequate response to these drugs, even after 3 months of regulated consumption, in favour of surgical excision which will be of both diagnostic and therapeutic value, as has been explained above.

In tubercular patient, there are several subpopulations of Mycobacterium tuberculosis with different rates of metabolic activity. Majority of the organisms are rapidly reproducing but some reproduce slowly or are semi-dormant with occasional spurts of metabolism. Therefore, combinations of multiple effective drugs, at correct dosage and for a prolonged period, are needed to treat this condition. Most of the extra pulmonary form of tuberculosis is generally treated with the standard six month regimen as required for pulmonary tuberculosis. In musculoskeletal tuberculosis more extended duration of treatment is required and is desirable; as there is affirmation that the standard six month treatment may not provide complete cure [24]. Therefore, rifampicin containing regimen of nine months or more duration is favoured by some authorities [25,26].

Nonetheless, appropriate duration of chemotherapy particularly in cases of tuberculosis of rib still remains unclear. On the basis of our experience and according to various other available literature [11,21,22,27], we propose at least a 12 month regimen of anti-tubercular medication with rifampacin in the doses of 10-15 mg/kg, isoniazid 5 mg/kg, ethambutol15 mg/kg and pyrazinamide 25 mg/kg of body weight for initial two months (induction therapy), followed by a ten month therapy with two drugs rifampacin and isoniazid (mantainance therapy) in the same dosage [22]. The majority of the organism are eliminated in the first two months of intensive phase but for achieving complete cure and to decrease the chances of recurrence, it is advisable to continue rifampicin and isoniazid for ten months. It may be wise to add injection streptomycin (15mg/kg as intramuscular injection and not more than 1 gm/day) in the induction phase, especially in chronic sufferers and immunocompromised patients.

5.Figures

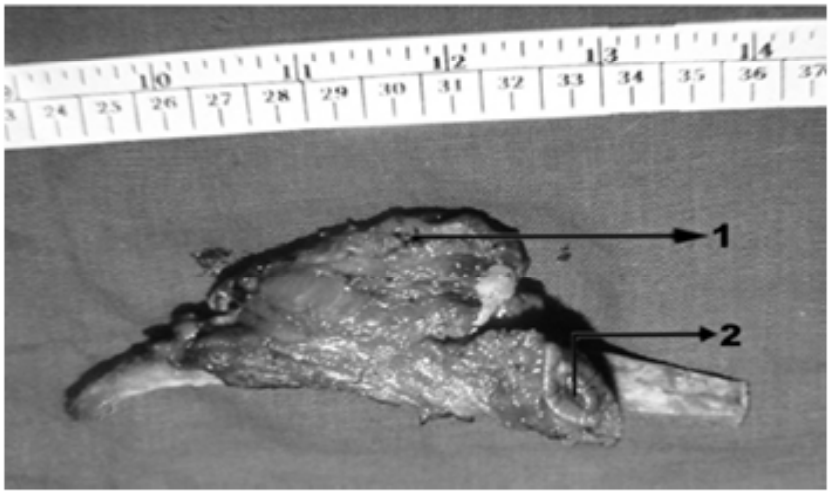

Figure1: Excised specimen of tubercular rib showing granulation tissue on the inner surface (1) and sinus opening on the outer surface (2).

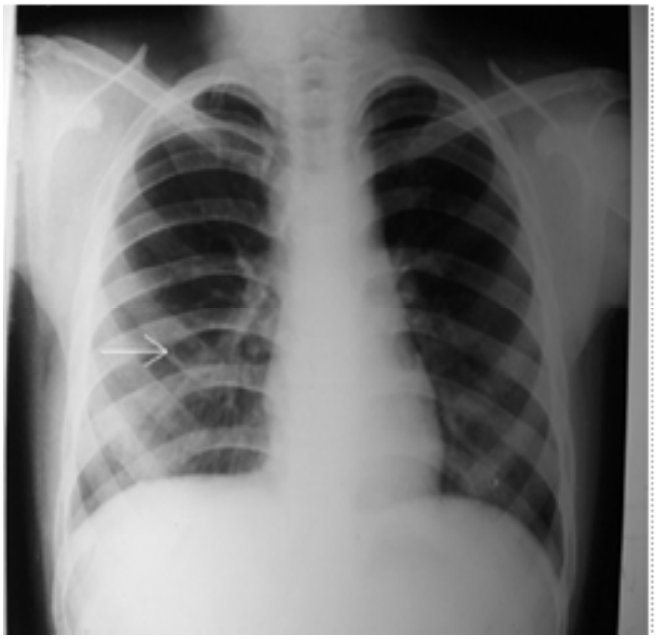

Figure 2: Osteolytic lesion of rib as seen in plain chest x-ray (arrow)

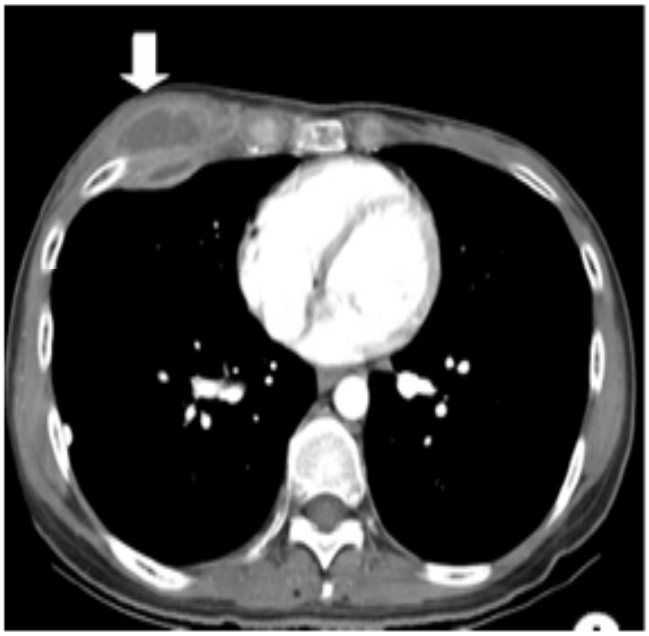

Figure 3: Chest CT showing soft tissue mass with central low density and with peripheral rim enhancement in the inner and outer sides of ribs (arrow).

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Diagnosis and Management of Tuberculosis

Chapter 3

Vaccination against Tuberculosis

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1. Introduction

Moving ahead of the World Health Organization Millennium Development goal targets for the tuberculosis (TB) set up till year 2015, the Sustainable Development Goals (SDGs) adopted by the United nations (UN) in 2015 has set one more goal i.e.point 3.3, as to eradicate the TB epidemic by the year 2030 [1]. Similarly,the World Health Assembly approved the WHO strategy to eradicate the TB in 2014 . The strategy aims for 95% reduction in TB deaths and 90% reduction in TB incidence rate by 2035 [2].

As per the WHO reports, an estimated 10.4 million cases occurred in 2015 including the 1.2 million people living with HIV/AIDS (PLHA). There were 1.8 million deaths due to TB of which 0.4 million were PLHAs. An estimated 480,000 developed multi drug resistant TB (MDR-TB) and an additional 100,000 were rifampicin resistant TB amenable for the second line treatment in 2015 [2].

Above data and the very fact that an open case of TB will infect 10-15 contacts leadsto an inference that despite the growth in diagnostic and therapeutic modalities, we may not be able to achieve the elimination of TB by 2050 [3]. This necessitates the availability of effective vaccines for TB control.

Till date Bacilli Calmette-Guerin (BCG) is the only available vaccine for TB. Since 1921 it has been administered to over 4 billion individuals, and almost 120 million children receive BCG annually [4]. BCG has limitations in terms of its efficacy in pulmonary TB while there are also safety concerns in the era of HIV. Therefore, there has been a need for a more effective and safer TB vaccine across the world. The fourth Global Forum for TB Vaccines in

the year 2015 witnessed at least 15 TB vaccine candidates in the clinical trials as compared to its first forum in 2001, when there was not a single candidate in worldwide clinical portfolio[5,6].

Multiple vaccine development strategies are the need of hour and can be simply grouped in one of the following:First option is toadminister prior to exposure to prevent the initial infection, next option is to give the vaccince after exposure, to individuals who are infected but may be asymptomatic and at a risk to develop the disease in future. This will protect them against disease manifestation and therefore transmission. Finally it can be given after the treatment of active disease to prevent reactivation and subsequent transmission [7,8].

Accordingly, currently available BCG and the vaccines in the clinical trials can be grouped into one of the three categories:

a. Priming vaccines: This type of vaccineis intended to prevent TB infection and the disease in infants who have not been infected with the TB bacilli. BCG comes under this category.

b. Booster Vaccines: This type of vaccine is are to be delivered during adolescence to prevent infection or to prevent latent infection to be developed into active disease as the BCG protection wanes.

c. Immunotherapeutic vaccines: This type of vaccine is are given to individual with active TB along with the TB chemotherapy intended to shorten the duration of therapy and/or prevention of recurrence after treatment [8].

2. Bacilli Calmette-Guerin (BCG)

It was obtained by Albert Calmette and Camille Guerin sustained efforts of 230 in vitro passages of strain of Mycobacterium bovis over a period of 13 years. It is a live attenuated vaccineand was first administered orally to an infact in 1921. Currently it is recommended as a single intra-dermal dose at the site of insertion of deltoid, in the healthy babies as close to the time of birth as possible in countries where TB is common. This is in accordance to the World Health Organization (WHO) recommendation for the children born in countries with TB as endemic disease. This measures leads to the protection against miliary TB and tubercular meningitis during childhood.Evidences do not support additional booster dosing. Adults who do not have tuberculosis and are not previously immunized and are frequently exposed to tuberculosis may get the immunization [9,10] Babies diagnosed of having HIV/AIDS should not be vaccinated [11].

Many countries have different immunization policies. India and Pakistan were the first countries outside the Europe to adopt universal BCG immunization policies since 1948, while the United States and Netherland have never adopted it as a routine policy [12]. United King-

dom has scrapped its use as a routine immunization in 2005 because of decreasingcost effectiveness secondary to decreasing annual incidences of TB [13].

Protective efficacy of BCG varies widely.A 1994 systemic review concludes that the BCG offers a protection of over 50% against getting TB.14 But this efficacy falls, as one approaches the areas near equator [14,15] According to one review in 2014, reduction in infection rate was between 19-27% while reduction in progression to active TB was by 71% [16]. Importantly the vaccine has positive effects against the severe forms of childhood TB, especially the meningeal and miliary TB. As per one meta-analysis, the BCG vaccination in the year 2002 reduced one case of tubercular meningitis for every 3435 vaccinations and one case of miliary Tb for every 9314 vaccinations, and it was deemed to be highly cost effective in endemic region [17]. There occurs a great deal of variations in terms of protection against pulmonary TB which is the most contagious form of TB. Clinical data have shown it to be anywhere between nil to 80% [14,1]. The differences in effectiveness of the vaccine has been attributed to multiple reasons including proximity to equator, genetic differences in the host populations, exposure to other bacterial infections, laboratory conditions including the strain being cultured and the growth media used [15,19].

The data about the duration of protection is highly inconsistent. British MRC study showed that the protection waning occurred to 59% subjects after 15 years and to zero subject after 20 years; while another study analyzing the evidence of protection in native Americans immunized in 1930s found vaccine efficacy to be 52% without statistically significant waning over 50-60 years of time [20,21].

BCG has happened to be a safe vaccine in healthy infants. Local site reactions including pain and scarring may occur. Regional suppurative or non-suppurative adenitis may happen and are self-limiting. Suppurative case may sometimes require needle aspiration or surgical excision [22,23]. Sometimes breast or gluteal abscess or regional osteomyelitis or osteitis may happen which may be potentially life threatening needing anti-tuberculous treatment [24]. Disseminated BCG infection (BCG-osis) though rare i.e. less than one per million immunization, may happen if the vaccine is given accidentally to an immuno-compromised patient e.g. in SCID patients [25]. The same may happen with HIV-infected children, even if the child is asymptomatic at the time vaccination [26,27]. Therefore, WHO has stopped recommending the BCG in the children with HIV [11].

Apart from the TB, BCG may find its use in leprosy, buruli ulcers and bladder and colorectal malignancies. In leprosy protective efficacy has been 26-41% in controlled trials to as high as 60% in case control and cohort studies though it is not specifically used to control leprosy [28,29,30,31]. Also, an added benefit of BCG vaccination in developing countries has been the provision of non-specific protection, what is called as heterologous effect or off-target

effect, against sepsis and respiratory infections in neonates.

3. Other Priming Vaccines in Pipeline

3.1. VPM1002

It is a recombinant BCG strain expressing membrane perforating listeriolysin which is encoded by the gene *hly* derived from Listeria monocytogenes. This strain lacks the urease C gene and has a hygromycin resistance marker [32]. The candidate vaccine showed a better protective effect in animal model as compared to the parental BCG. In its phase 1 trial it was noted safe to be administered in healthy adults and had similar adverse effect profile in comparison to BCG and had no human to human transmission. It induced goodCD4+ and CD8+ T cell responses [32,33]. Phase 2 trial to study its effects in newborn is on the way [34].

3.2. MTBVAC

This vaccine has the credit of being the first live attenuated Mtbvaccine to be entered in Phase I study. MTBVAC is a new vaccine strain having mutations in phoP and fadD26 genes. fadD26 translational product is required to synthesize phthiocerol dimycocerosates, a cell wall content that protect the bacilli from host defenses. Following the promising Phase 1 results, it is now in the Phase 2a trial [35,36].

4. Therapeutic Vaccines

4.1. RUTI

This is an immunotherapeutic vaccine composed of detoxified liposomal fragments of Mycobacterium tuberculosis. It is supposed to improve the treatmentof latent Tb after a short course of anti-tubercular treatment. In its animal model it showed a good safety profile and elicited TH1, TH2, TH3 , CD8+ T cells and antibodies responses. In Phase 1 trial it was well tolerated after being given subcutaneously in BCG naïve healthy adults. There were local reactions reported, without any serious adverse events. It also induced specific cellular and antibody based responses [37,38]. Now it is in Phase 2a trial.

4.2. Mycobacterium vaccae

This is a whole cell inactivated Mycobacteriumvaccae (MV) meant to be administered intra-dermally. A Meta-analysis showed that MV when added to the anti-tubercular chemotherapy in TB patients who have received no treatment earlier showed improvement in sputum conversion and X-ray picture. Another meta-analysis also revealed that it was able to prevent the TB in high risk categories and is safe and immunogenic in people living with HIV/AIDS. This can have a role as prophylactic vaccine in HIV infected patients. Phase 3 trial is going on and has shown to induce a variable IFN- γ response [39,40].

5. Virus Based Booster Vaccines

Viral vector based vaccine technology has an ease of cloning large or multiple immune-dominant antigens and can be exploited for a high titer scale up production. Vaccinia, a wild type pox virus qualifies the scenario but has safety issue in HIV subjects due to its high replicating capacity. A Modified vaccinia virus Ankara (MVA) has been made replication deficient retaining its ability to express protein. Adenovirus also hasa type1 immuno-adjuvant property and has been rendered replication deficient by deletion of gene E1. This has an additional advantage of natural respiratory tropism but is limited by neutralizing antibodies in human plasma [41,42].

5.1. MVA85A

Here mycobacterial antigen 85A is delivered by MVA delivery system. It was found to improve the BCG induced protection in animal model. In Phase I trial in UK in healthy adults including BCG vaccinated adults and subjects with LTBI, the results regarding safety and immunogenicity were promising and further trials are on the way [43,44,45]. It has additional advantage of demonstrating safety in HIV infected individuals or HIV-TB co-infection and infants [46]. Also the frequency of antigen specific cells remain significantly higher from the baseline at least for a year [47]. In its Phase 2b clinical trial, done on 2797 HIV-negative healthy infants of four to six months and previously vaccinated with BCG, the vaccine met the safety objectives but the TB rates in vaccinated children was not statistically significant as compared to the placebo. Thus the results described were disappointing in terms of preventing TB in BCG vaccinated infants [48].

5.2. AERAS402/CrucellAd35

AERAS is a replication deficient adenovirus 35 expressing the mycobacterialantigens 85A, 85B and TB10.4. Ad35 offers an advantage of low level of immunity, and hence the pre-existing neutralizing antibodies. Clinical trials after their promise in Phase1 trials, regarding the safety and efficacy are on the way in BCG vaccinated subjects in both HIV infected as well as HIV non-infected population [49,50,51].

5.3. Ad5Ag85A

This utilizes the Ad5 virus to express mycobacterial antigens 85A. The vaccine showed good results in mice and guinea pigs when administered intranasal but not intramuscularly. Currently in the clinical trials, the vaccine has the problem of facing pre-existing neutralizing antibodies as well as concern of acquiring HIV infection while vaccinating the Ad5 seropositive individual in the STEP trial [52,53,54].

Others MVA85A based viral vector in phase 1 evaluation are the simian virus based vac-

cine ChAdOx1.85A+ MVA85A and MVA85A-IMX313 [55].

Another initiative currently in phase1 is the live influenza vector expressing the mycobacterial antigen Ag85A and ESAT6 [55].

6. Protein Adjuvant Vaccines

Protein has the advantage of production in bulk but requires some agent called adjuvants to induce immune responses of desirable potency and durability. These agents can be antigen delivery systems like aluminium based adjuvants or may be immunopotentiating agents like toll-like receptor ligands, cytokines or the bacterial toxins. Recent development of immune-stimulating adjuvants have made the TB protein subunit vaccines a feasible approach and many are in the clinical trials too [56].

6.1. M72AS01

M72 is related to Mtb72F protein which is a fusion protein comprising up of Mtb39a and Mtb32a antigens. A point mutation carried out in the Mtb32a improved the long term stability of vaccine. AS01E adjuvant system contains the immunostimulant MPL and QS2 combined with liposome to induce humoral and TH1 cellular responses [57,58].

The vaccine was well tolerated and immunogenic in adults with or without previous exposure to BCG or Mtb. Concerns regarding the effectiveness are there owing to strain related variations in antigen sequences. Unlike MVA85A, CD8+ T cell response was not seen with this preparation [57,59]. Local adverse events usually resolved within a week and may have been due to the adjuvant.Phase 2 trial is on the way including the HIV and TB individuals.

6.2. Hybrid 1 + IC31

This is a recombinant subunit vaccine where antigens ESAT6 and Ag85B are adjuvanted with IC31 system (polyaminoacid KLK and oligodeoxynucleotide ODN1a). It has been shown to provide durable TH1 responses in mycobacterially naïve subjects, BCG vaccinated individuals or previously TB infected adults. Though a week immune response is seen against ESAT6, but had a more potent protective effect than the Ag85B alone vaccine. The vaccine has been safe. Few local andsystemic events were milder and resolved in less than 48 hours. No serious adverse events have been reported. Concern regarding the interference of vaccine with Interferon Gamma Release Assay (IGRA) test due to ESAT6 involvement in few subjects needs further studies to validate [60,61,62].

6.3. Hybrid 4 +IC31

This vaccine was developed on the same line as H1/IC31, but the antigen ESAT6 was replaced by TB10.4 to avoid IGRA related interferences. The vaccine is in the Phase 2 trial

evaluation and is a good candidate in pipeline [63,64].

6.4. Hybrid 56 + IC31

H56 is a fusion protein of Ag85B, ESAT6, and the latency associated protein Rv2660c. The vaccine uses this H56 combined with the adjuvant IC31. It was safe and showed excellent control on latent infection in non-primate model. Moreover, in vaccinated monkeys anti-TNF antibodiesdid not induced reactivation of latent Tb [65]. In its first human Phase 1 trial the vaccine induced polyfunctional (IFN γ+, TNF α+, IL2+) CD4+ T cells) response, that too after the low doses. CD45RA-CCR7+ central memory phenotype cells were also expressed.No serious adverse events were reported though few subjects had transient cardiovascular events [66].

6.5. ID93/GLA-SE

This vaccine uses ID93, a fusion protein of four Mtb antigens Rv2608, Rv3619, Rv3620 and Rv1813 adjuvanted with glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE). It induced a significant multifunctional CD4+ response in BCG vaccinated or nonvaccinatedmice and guinea pigs. In non-human primates it was well tolerated and induced both TH1 and TH2 type responses [67]. Moreover it also protected from MDR-TB in animal models [68]. Clinical trials regarding safety and efficacy in human is ongoing [69,70].

7. Whole Cell Inactivated Vaccine

7.1. DAR-901

It is an inactivated whole cell non-tuberculous mycobacterial vaccine from the master cell bank of previous vaccine SRL-172. In its phase1 trial with participants who were BCG primed and included HIV positives- negatives and IGRA positives-negatives, it induced both cellular and humoral responses and had acceptable safety and tolerability profile [71]. Currently the phase 2 trial is ongoing [72].

8. Conclusion

It is indeed hopeful to have a number of vaccine candidates in the active phases of development for this dreaded disease, TB. The search will continue and may get modified with time till an ideal TB vaccine is available to make the epidemiology of the disease favorable, including the drug resistance and its nexus with the HIV. Till then, we have to rely on BCG especially for the prevention of serious manifestations of TB in pediatric population and preventing the mortality.

Table 1 : BCG and other vaccine candidates.

Name	Type	Composition	Status
Priming vaccines			
BCG	Live attenuated	Live attenuated *Mycobacterium bovis*	In use since 1921
MTBVAC	Live attenuated	Live attenuated *Mycobacterium tuberculosis*	Phase IIa
VPM1002	Live attenuated	Live attenuated recombinant bacille Calmette-Guerin (r-BCG)	Phase IIa
Booster Vaccines			
M72/AS01	Protein/Adjuvant	Mtb39a-Mtb32a fusion protein with AS01 adjuvant	Phase IIb
Hybrid 4 + IC31	Protein adjuvant	TB10.4-Ag85B fusion protein with IC31 adjuvant	Phase II
Hybrid 56 + IC31	Protein Adjuvant	Ag85B-ESAT6-Rv2660c fusion protein with IC31 adjuvant	Phase IIa
Hybrid 1 +IC31	Protein Adjuvant	ESAT6-Ag85B fusion protein with IC31 adjuvant	Phase IIa
ID 93+ GLA-SE	Protein Adjuvant	Rv2608-Rv3619-Rv3620-Rv1813 fusion protein with GLA-SE adjuvant	Phase I
Ad5Ag85A	Viral Vector	Recombinant adenovirus expressing Ag85A	Phase I
Crucell Ad35+ MVA85A	Viral Vector	Recombinant adenovirus 35 expressing Ag85A-Ag85B-TB 10.4 fusion protein	Phase IIa
ChAdOx1.85A+ MVA85A	Viral Vector	Simian virus ChAdOx1.85A plus MVA85A	Phase I
MVA85A	Viral Vector	Modified Vaccinia virus Ankara expressing Ag85A	Phase IIb
MVA85A-IMX313	Viral Vector	MVA85A combined with carrier protein IMX313	Phase I completed
TB/FLU-04L	Viral Vector	Live recombinant influenza vectored tuberculosis vaccine expressing Ag85A and ESAT 6	Phase I
Dar-901	Inactivated whole cell vaccine	Whole cell *M.obuense*	Phase I
Immunotherapeutic Vaccines			
M. vaccae	Inactivated Whole cell vaccine	Whole cell *M.vaccae*	Phase III
RUTI	Fragmented M.tuberculosis	Detoxified liposomal fragments of *M.tuberculosis*	Phase IIa

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Diagnosis and Management of Tuberculosis

Chapter 4

Treatment response of retreatment category tribal Pulmonary Tuberculosis patients lived in Eastern India

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Abstract

Objective: The study was conducted to assess the treatment outcome of different category retreatment cases with the aim of finding out the important predictors of unfavorable outcomes.

Methodology: This hospital based prospective cohort study was conducted in three Tuberculosis unit (TU) of west Midnapore (a district of eastern India), covering mostly the tribal populated areas. Patients who were registered for Category II anti-tuberculosis treatment between 1st quarter 2013 (Jan to Mar) to 4th quarter 2013 (Oct to Dec), were considered as our study cohort and they were followed upto December 2014. The study was started with 177 patients but ultimately ended with 165 patients.

Results: Unfavorable outcome was observed among 24.8% patients. Among them mostly (51.2%) were defaulter, 22% were failure case and 26.8% patients died during treatment. Patients, who were minority by religion, were found 4 times more vulnerable for unfavorable outcome. Unfavorable outcome were found 7 times more common among retreatment TB cases who remain sputum positive after completion of initiation phase of category II treatment.

Conclusion: Programmatic approach should be specified to address the minority by religion population and to reduce the load of sputum positive cases after completion of initiation phase treatment by tracking them.

Keywords: tribal population; retreatment pulmonary TB case; sputum positive; prospective cohort study

1. Introduction

In Indian continent Tuberculosis (TB) is oldest communicable, fatal infectious disease that is deeply rooted among Indian population since 1500 BCE (well evident around 500 BCE literatures). History has evidence that many emperors died and many empire demolished due to this fatal disease in India [1,2]. Still India can't rid of from this curse.

1.1 Current situation

The World Health Organisation (WHO) statistics of 2015 reported that only in India Tuberculosis incidence is near to one fourth of global TB incidence (2.2 million cases of TB for India out of a global incidence of 9.6 million). Prevalence of TB was 2.5 million estimated in same year. But the burden of Tuberculosis infection is huge. About 40% people of India is carrying this micro-organism in their body. Most of them have latent infection rather than active manifestation. It indicates that number of susceptible population is vast in this country [3,4]. Globally 2–3 million deaths are reported annually out of more than 9.8 million new cases of active TB [5,6]. In 2014 [1], 78,000 retreatment pulmonary TB cases were registered in India, 70% were treated successfully and 8% were died [7].

After completion of standard first-line TB treatment patients who are failure, defaulter, or relapse, are grouped together as Category II cases and allocated for retreatment according to the World Health Organization (WHO). In India where individual drug susceptibility testing (DST) facilities are not universally scaled up still now, there patients are often treated with a standard retreatment regimen of first-line agents (a regimen that adds a single drug to the standard initial TB treatment regimen) [8]. Retreatment case's outcomes often are found poor as MDR-TB, especially in patients with treatment failure or default cases [9].

Though in late, but India realized to face the emergent problem immediately and launched Revised National Tuberculosis Control Programme (RNTCP) in 1997. After that, this programme gradually expanded across the country and in 2011, it achieved initial targets (71% new sputum positive case detection rate and 87% treatment success rate). So many programmatic errors, HIV-TB treatment issues, increasing number of Multi drug resistant TB cases are hindering the success over Tuberculosis [10].

Inappropriate implementation of the Revised National Tuberculosis Control Programme (RNTCP) causes precipitation of MDR-TB cases in the community. In this situation, India is not well equipped to prevent the propagation and dissemination of MDR-TB cases. So a new reemerging threat is slowly growing within the Indian population that may arise as a big challenge in future. MDR-TB is a man-made phenomenon-poor treatment, poor drugs and poor adherence lead to the development of MDR-TB [11].

1.2 Why we concern about tribal populations?

8.6% population of India belongs to tribal group and they are considered as an under privileged society due to lack of knowledge, poverty, ancient under developed culture, life style and behaviors. Prevalence of Tuberculosis among tribal population is varied in different studies. Beena Thomas et.al. estimated pooled TB prevalence among Indian Tribal Population (703 per 1 lakh) and that was significantly higher than estimated figure of TB prevalence of all over India (256 per 100,000) [12]. But Bhat J et al found no significant difference of TB prevalence among tribal and non tribal groups [13]. Most of the studies among tribal population in this field has been published on prevalence of the disease. Predictors of the disease or response of treatment among them got less attention in the research field.

The tribal populations of Eastern India are likely to live in particular discrete hard to reach geographic areas and always remain away from the light of civilization and often with poor access to the health care system. These factors make a communication barrier with all health care facilities and make them more vulnerable to develop drug resistant TB.

Smear +ve and –ve previously treated pulmonary TB cases are suspected as MDR-TB case according to Programmatic Management of Drug Resistant TB (PMDT) guideline. But in India where drug resistance TB diagnosis facilities are not available widely still now, there unfavorable outcomes of retreatment TB cases in the environment of poor RNTCP covered area (Tribal areas) can be suspected highly as the source of drug resistant TB. Unfavourable outcome of retreatment TB cases and poorly accessed health facility areas (Tribal area) both could be consider as common attributed factors for drug resistant TB [11].

In eastern India retreatment TB related research work among tribal population is very limited. In this background a study was conducted to assess the treatment outcome of different category retreatment cases with the aim of finding out the important predictors of unfavorable outcomes and enlighten the issues which are paid less importance.

2. Materials & methods

A prospective cohort study was conducted in three Tuberculosis units (TU) of west Midnapore (a district of eastern India), covering mostly the tribal populated areas. West Midnapore is a district of eastern India where 14.87% populations are belonging to tribal family and are living with their traditional tribal culture. In this rural district tuberculosis treatment services are provided from 11 TUs, 52 district microscopy centre, 119 peripheral health institute, 25 sputum collection centre and 945 DOT centers. 25% of TUs (3 TU) were purposively selected where tribal inhabitants are more.

Our study cohort included all the tribal people who were registered for Category II anti-

tuberculosis treatment between 1st quarter 2013 (Jan to Mar) to 4th quarter 2013 (Oct to Dec). They were followed up until their treatment was completed as per DOTS guideline that is December 2014. Patients, who had incomplete follow up data in register and who were transferred out during the treatment, were excluded from the study. All the data related to exposure and outcomes were collected from Tuberculosis unit's register and their follow up data were also tracked from same secondary data source.

Complete enumeration method was applied here. In the year of 2013, 478 retreatment cases were registered in those 3 TUs. Among them 37% (177) cases were belonging to tribal population. Tribal were identified, by observing the surname lists under the tribal caste with clarification from the General Administration, as and when necessary. The study was started with these 177 patients but ultimately ended with 165 patients, because 9 patients were excluded due to incompletion of data and 3 were transferred out during the treatment. (Figure 1).

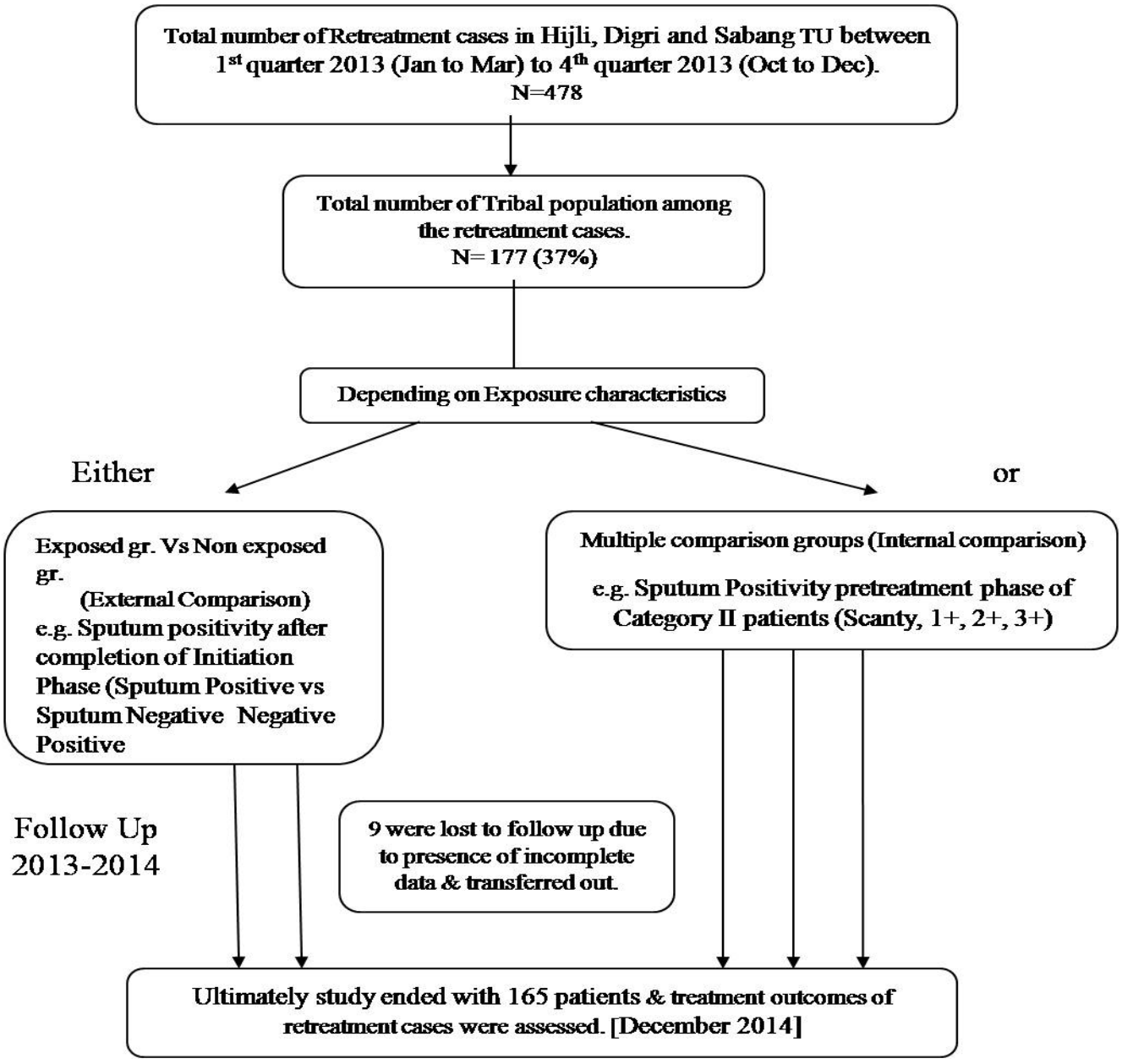

Figure 1: Schematic diagram showing the study design.

3. Operational definitions according to RNTCP guideline [14]:

3.1 Category of patients present among retreatment case cohort

Relapse: A TB patient who was declared cured or treatment completed by a physician and who reports back to the health facility and is now found to be sputum smear positive. *Treatment after default*: A patient, who has received treatment for TB for a month or more from any source and returns for treatment after having defaulted i.e., not taken anti-TB drugs consecutively for two months or more and found to be smear-positive. *Treatment failure*: Any TB patient who is smear-positive at 5 months or more after initiation of treatment. *Others*: A patient who does not fit into the any of the types mentioned above. The reasons for labeling a patient under this type must be specified in the Treatment card and TB Register.

3.2 Treatment outcome

3.2.1 Favourable outcome

Cured: Initially sputum smear positive patient who has completed treatment and had negative sputum smears on two occasions, one of which was at the end of the treatment. *Treatment completed*: Initially sputum smear positive patient who has completed treatment with negative smears at end of the intensive phase / two months in the continuation phase, but none at the end of the treatment is declared as treatment completed. Or initially sputum smear negative patient who has received full course of treatment and has not become smear positive at the end of the treatment.

3.2.2 Unfavourable outcome

Died: Patient who died during the course of treatment regardless of any cause. *Failure*: Any TB patient who is smear positive at five months or more after initiation of the treatment and not put on MDR-TB treatment. *Defaulted*: A Patient after treatment initiation has interrupted treatment consecutively for >2 months.

Patients were categorized according to following variables like age, gender, address, religion, type of category II patient (relapse, failure, treatment after default), level of sputum positivity. These variables were considered as exposure characteristics for this cohort.

Statistical analysis: Data were entered in Microsoft Excel worksheet (Microsoft, Redwoods, WA, USA) and were analyzed using IBM SPSS software, version 19.0 (Statistical Package for the Social Sciences Inc, Chicago, IL, USA) and Microsoft Excel. Chi square test was performed for bivariate analysis. Variables which were found statistically significant (p= 0.05) in bivariate analysis were considered for logistic regression model and adjusted odd ratio was assessed for predictor variables. Relative risk and attributed risk were also assessed for

significant predictors of unfavourable outcome.

4. Results

Baseline characteristics of the cohort: Among 165 tribal patients, 53.9% were belonging to adolescent and young adult group, 84.2% were male, 81.8% were Hindu and 69.7% were residing at rural community. Most of the cases of Category II patients were relapsed case (33.9%) and 29.7% cases were defaulter after preliminary treatment. In pretreatment period, most of the cases (31.5%) were 2+ sputum positive. After receiving initiation phase of category II treatment 9.7% remained sputum positive. Unfavorable outcome was observed among 24.8% patients. Among them mostly (51.2%) were defaulter, 22% were failure case and 26.8% patients died during treatment.

Bivariate analysis: As all the variables (exposure characteristics) were qualitative in nature, chi-square test/ Fisher exact test were applied to find out the association with treatment outcomes. The associations of unfavorable outcomes were found significantly more among adolescent and young adult patients (73.2%), treatment after default category patients (46.3%), patients who were 2+ & 3+ sputum positive at pretreatment phase (39% + 31.7% = 70.7%) and who were sputum positive after completion of initiation phase (75.6%). The chances of unfavorable outcomes were observed significantly more among minority by religion patients in comparison to favorable outcomes (34.1% vs 12.9%) (Table 1).

Table 1: Bivariate analysis between patient's profile and treatment outcome.

Patient's Profile	Favourable outcome N(%)	Unfavourable outcome N (%)	Chi-square test
Age			
Adolescent & Young Adult (11-40)	59 (47.6)	30 (73.2)	χ^2=10.066 df=2 p=0.007*
Middle age (41-60)	59 (47.6)	8 (19.5)	
Geriatric >60	6 (4.8)	3 (7.3)	
Gender			χ^2=0.052
Male	104 (83.9)	35 (85.4)	df=1
Female	20 (16.1)	6 (14.6)	p=0.82
Address			χ^2=0.028
Rural	86 (69.4)	29 (70.7)	df=1
Urban	38 (30.6)	12 (29.3)	p=0.868
Religion			χ^2=9.838
Hindu	108 (87.1)	27 (65.9)	df=2
Minority by religion	16 (12.9)	14 (34.1)	p=0.007*

Type of Category II patients **Treatment after Default** **Failure** **Relapse** **Transferred In** **Others**	 30 (24.2) 6 (4.8) 43 (34.7) 35 (28.2) 10 (8.1)	 19 (46.3) 5 (12.2) 13 (31.7) 2 (4.9) 2 (4.9)	χ^2=15.591 df=4 p=0.004*
Sputum Positivity pretreatment phase of Category II patients **Scanty** **1+** **2+** **3+**	 47 (37.9) 27 (21.8) 36 (29) 14 (11.3)	 3 (7.3) 9 (22) 16 (39) 13 (31.7)	χ^2=18.338 df=3 p=0.000*
Sputum positivity after completion of Initiation Phase **Negative** **Positive**	 118 (95.2) 6 (4.8)	 31 (75.6) 10 (24.4)	Fisher exact test p value= 0.001*

Among all types of unfavorable outcomes, defaulters were found as most common treatment outcome (51.2%). Defaulted treatment outcomes were observed mostly among adolescent and young adult patients (53.3%), minority by religion (71.5%) and treatment after default category II patients (52.6%). After completion of initiation phase 50% of the sputum positive patients completed their treatment period as failure cases. About half (46.1%) of the pretreatment phase (3+) sputum positive patients, died during category II treatment phase. (Table 2).

Table 2: Distribution of tribal patients according to the patient's profile and treatment outcome.

	Favourable outcome			**Un-favourable outcome**			
	Cured	**Treatment Completed**	**Total**	**Failure**	**Defaulted**	**Died**	**Total**
Age							
Adolescent & Young Adult (11-40)	41 (69.49)	18 (30.51)	59 (100)	6 (20)	16 (53.3)	8 (26.7)	30 (100)
Middle age (41-60)	38 (64.4)	21 (35.6)	59 (100)	2 (25)	3 (37.5)	3 (37.5)	8 (100)
Geriatric >60	3 (50)	3 (50)	6 (100)	1 (33.3)	2 (66.7)	0 (0)	3 (100)
Gender							
Male	71 (68.3)	33 (31.7)	104 (100)	8 (22.9)	17 (48.5)	10 (28.6)	35 (100)

Female	11 (55)	9 (45)	20 (100)	1 (16.7)	4 (66.6)	1 (16.7)	6 (100)
Address							
Rural	55 (64)	31 (36)	86 (100)	7 (24.1)	16 (55.2)	6 (20.7)	29 (100)
Urban	27 (71.1)	11 (28.9)	38 (100)	2 (16.6)	5 (41.7)	5 (41.7)	12 (100)
Religion							
Hindu	68 (63)	40 (37)	108 (100)	8 (29.6)	11 (40.6)	8 (29.6)	27 (100)
Minority by religion	14 (87.5)	2 (12.5)	16 (100)	1(7.1)	10 (71.5)	3 (21.4)	14 (100)
Types of Category II patient							
Treatment after Default	29 (96.7)	1(3.3)	30 (100)	3(15.8)	10 (52.6)	6 (31.6)	19 (100)
Failure	6 (100)	0 (0)	6 (100)	3(60)	2(40)	0 (0)	5 (100)
Relapse	36 (83.7)	7(16.3)	43 (100)	2(15.4)	7(53.8)	4(30.8)	13 (100)
Others	8(80)	2(20)	10 (100)	1(50)	1(50)	0(0)	2 (100)
Transferred In	3(8.6)	32(91.4)	35 (100)	0(0)	1(50)	1(50)	2 (100)
Sputum Positivity pretreatment phase of Category II patients							
Scanty	10 (21.3)	37 (78.7)	47 (100)	1 (33.3)	1 (33.3)	1 (33.3)	3 (100)
1+	25 (92.6)	2 (7.4)	27 (100)	1 (11.1)	6 (66.7)	2 (22.2)	9 (100)
2+	33 (91.7)	3 (8.3)	36 (100)	4 (25)	10 (62.5)	2 (12.5)	16 (100)
3+	14 (100)	0 (0)	14 (100)	3 (23.1)	4 (30.8)	6 (46.1)	13 (100)
Sputum positivity after completion of Initiation Phase							
Negative	77 (65.3)	41 (34.7)	118 (100)	4 (12.9)	19 (61.3)	8 (25.8)	31 (100)
Positive	5 (83.3)	1 (16.7)	6 (100)	5 (50)	2 (20)	3 (30)	10 (100)

Logistic regression: Factors which were found statistically significant in bivariate analysis were considered for logistic regression to measures the relationship between the categorical dependent variable (unfavorable outcome) and one or more independent variables. The logistic regression model was significant, as evident from omnibus chi-square test (P = 0.00). All the independent variables together can explain between 21.7 % to 32.1% variance of the dependent variable (unfavorable outcome), as evident from Cox & Snell and Nagelkerke R square. Regression model can correctly predict 91.9% of favorable outcome and 36.6% of unfavorable outcome. Overall, the model predicts 78.2% of the outcome correctly, as shown by classification table. Ultimately in logistic regression model, religion and sputum positivity after completion of initiation phase were predicted as the significant variable of unfavorable

outcome. Patients, who were minority by religion, were found 4 times more vulnerable for unfavorable outcome [Odd's Ratio = 4.1 (95% Confidence Interval = 1.6-10.8), P = 0.004]. Unfavorable outcome were found 7 times more common among retreatment TB cases who remain sputum positive after completion of initiation phase of category II treatment [Odd's Ratio = 6.9 (95% Confidence Interval=1.9-24.7), P=0.003] (Table 3).

Table 3: Logistic regression model for the predictors of unfavourable outcome

Omnibus Tests of Model Coefficients				
		Chi-square	df	Sig.
Step 1	Step	40.272	8	.000
	Block	40.272	8	.000
	Model	40.272	8	.000

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	144.747[a]	.217	.321

a. Estimation terminated at iteration number 6 because parameter estimates changed by less than .001.

Variables in the Equation									
		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	11. Age	-.014	.016	.723	1	.395	.986	.955	1.018
	2. Religion	1.415	.493	8.226	1	.004	4.115	1.565	10.821
	3. Type of Category II patients			6.099	4	.192			
	Treatment after Default(1)	1.416	.896	2.494	1	.114	4.120	.711	23.876
	Failure (2)	.985	1.080	.831	1	.362	2.678	.322	22.241
	Relapse (3)	.671	.898	.558	1	.455	1.955	.336	11.367
	Transferred in & Others(4)	-.529	1.226	.186	1	.666	.589	.053	6.515
	4. Sputum Positivity pretreatment phase of Category II patients	.358	.250	2.042	1	.153	1.430	.876	2.336
	5. Sputum positivity after completion of Initiation Phase	1.937	.649	8.920	1	.003	6.940	1.946	24.746
	Constant	-4.402	1.359	10.491	1	.001	.012		

a. Variable(s) entered on step 1: Age, Religion, Type of Category II patients, Sputum Positivity pretreatment phase of Category II patients, Sputum positivity after completion of Initiation Phase.

Classification Table[a]					
	Observed		Predicted		
			outcome		Percentage Correct
			.00	1.00	
Step 1	outcome	Favorable (0.00)	114	10	91.9
		Unfavorable (1.00)	26	15	36.6
	Overall Percentage				78.2

a. The cut value is .500

Scoring:

Religion: Hindu=0, Minority by religion=1; Type of Category II patients: Treatment after Default=0,

Failure=1, Relapse=2, Transferred in & Others=3; Sputum Positivity pretreatment phase of Category II patients: (Scanty & 1+) = 0, (2+&3+) =1; Sputum positivity after completion of Initiation Phase: negative=0, positive=1.

Relative risk and attributed risk: Relative risk and attributed risk were calculated for the significant predictors. Relative risk showed that patients, who had sputum positive after completion of initiation phase, had 2.98 times more risk than others for the development of unfavorable outcome. In case of minority by religion that risk was 2.35 times more than Hindu tribal. Attributed risk indicated that 66.4% of unfavorable outcome occurred due to failure of sputum conversion after initiation phase of treatment. Religion of retreatment cases attributed 57.4% cases of unfavorable outcome when they were belonging to minority by religion (Table 4).

Table 4: Relative risk and Attributed risk for significant predictors of unfavorable outcome

Predictors of unfavorable outcome	Relative risk	Attributed risk (%)
Religion	2.35	57.4
Sputum positivity after completion of Initiation Phase	2.98	66.4

5. Discussion

In the present study we have found 75.2% favourable outcome that is similar with the treatment success rate (71%) of India in retreatment TB cases [15].

Table 5: Comparison between different studies about treatment success rate among retreatment Tuberculosis cases

Study	Place of study	Population of study	Treatment Success rate
Our study, 2014	West Bengal, India	Tribal population	75.2%
Sevim T et al, 2002[16]	India	All type of relapse & defaulter cases	71.9%
Win A N et al, 2012[17]	Mayanmar	All type of retreatment cases	73%
Brahmapurkar KP[18]**, 2013**	Chattisgarh, India	All type of retreatment cases, mostly tribal patients	64.9%

Though the studies were conducted in different settings still it showed that treatment success rate among retreatment cases within India did not vary enough as well as in other Asian country (Mayanmar) also. But Brahmapurkar KP study conducted in tribal district of central India found lower success rate. It indicates that treatment success rate varies with tribal community. As treatment response of eastern India tribal retreatment patients was found comparable with overall performance of India.

Among the unfavourable outcomes more were defaulter (51.2%) that is also collaborate with the Vijay S et al (72.8%) & Vasudevan K et al (37.6%) study, but in our study, rate of defaulters were quite less than Vijay S et al study and quite higher than Vasudevan K et al study [19,20]. Though the rate of defaulters were varied widely within the India but in all studies it were counted as most common unfavourable outcome. More number of defaulters indicates the failure of programme implementation like poor coverage and lack of tracking activity of defaulters under the RNTCP. In the present study, unfavorable outcomes were found significantly more among adolescent and young adult patients (73.2%), minority by religion (26.8%), treatment after default category patients (46.3%), patients who were 2+ & 3+ sputum positive at pretreatment phase and who were sputum positive after completion of initiation phase. Vijay et al study found treatment after default category patients as potential defaulters/ unfavourable outcome that is also supported by our study findings. Both Vijay et al and Dandekar RH et al study found gender as significant predictors of overall unfavourable treatment outcome, but we did not find any significant relationship between gender and unfavourable treatment outcome [19,21]. We observed unfavourable outcome commonly among high grade sputum positive patients that is also collaborative with the Mukherjee A et al study observations. They also found unfavourable outcome most likely among treatment failure subgroup but we observed it more among treatment after default category patients (46.3%) [22].

Ultimately logistic regression model found that patients, who were minority by religion, were 4 times more vulnerable for unfavorable outcome [Odd's Ratio=4.1 (95% Confi-

dence Interval=1.6-10.8), P=0.004] and it was 7 times more common among retreatment TB cases who remain sputum positive after completion of initiation phase of category II treatment [Odd's Ratio = 6.9 (95% Confidence Interval = 1.9-24.7), P = 0.003]. This regression model can predict only 36.6% of unfavorable outcome correctly. Programmatic variables were not assessed in our study that can explain the low predictability of regression model. In spite of poor predicted ability of the regression model, it predicted two most important predictors of unfavourable outcome that should be intervened to improve the treatment outcome of retreatment TB cases.

In our study patients who were minority by religion they were more susceptible to unfavourable outcomes, but Dandekar RH found no significant relationship between them and no such observation was found in other studies also. It might be that minority among tribals were more hard to reach group due to their cultural and communication barrier. Sputum conversion rate in our study was 90.3% and that is quite higher than Vasudevan K et al study's observation (76.9%). Patients who remain sputum positive after completion of initiation phase of category II treatment they were found more susceptible to unfavourable outcome [OR=6.9] that is also suggested by Dooley KE et al study [OR=7.14] in Morocco [23]. In our study, although there was no such scope to measure drug sensitivity and resistance, it could be assume that more unfavourable outcome among unsuccessful sputum converted cases probably due to development of drug resistance. It might be single drug or multi drug resistance. If the MDR-TB diagnostics resources facility scaled up everywhere then the problem could be figure out more elaborately and results would be more evidence based. Kritski et al reported that unfavorable response to retreatment regimen was significantly associated with multidrug-resistant M *tuberculosis* infection [24].

Though the present study was not any comparison between tribal and non tribal population, its outcome were found more or less same as overall treatment outcome. Although poor living conditions, malnutrition, erroneous health assumptions and beliefs concerning TB, lack of resources and treatment by traditional healers increases the burden of TB among the tribal/indigenous population [25-27], but the treatment outcome is not significantly associated with ethnicity that was also observed in a previous study conducted by Chakrabarti S et al in West Bengal [27]. It indirectly indicates that programme failure related variables like lack of access to proper treatment, traditional and cultural barriers, lack of tracking of defaulters, etc. which were not addressed in previous study, were more important predictors than socio-demographic characteristics and it was felt by Chakrabarti et al. also [28]. That means unfavourable outcome among tribal population can be avoided, because it less depends on non modifiable factors like ethnicity.

6. Conclusion

This study was conducted among tribal population in assumption that unfavourable outcome will be found more prevalent among them. But the study results collaborated with the overall treatment outcome of India. In the end of the study patients who were minority by religion and who remained sputum positive after completion of initiation phase of treatment, were found more susceptible to unfavourable outcome. Programmatic approach should be specified to address the minority by religion population by breaking the communication barrier. Tracking of defaulters, early diagnosis of drug resistance cases by scaling up diagnostic facilities can reduce the load of sputum positive cases after completion of initiation phase treatment as well as unfavourable outcome also. The study concluded that tribal ethnicity does not affect retreatment case treatment outcome, but their religious belief have an impact on it. In future studies if the programme related variables could be addressed, more important predictors of retreatment cases outcome will be revealed.

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Diagnosis and Management of Tuberculosis

Chapter 5

Tuberculosis and its Diagnosis: A Review

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1. Introduction

Mycobacterium genus covers more than 140 species, these species are divided into three major sets, that is, *M.tuberculosis complex* (MTBC), *M.leprae*, and mycobacteria other than tuberculosis(MOTT) [1]. The MTBC comprises of five species including *M.tuberculosis, M.bovis*, *M.africanum*, *M.canetti*, and *M.microti* that causing tuberculosis (TB) [2]. *M.tuberculosis*, is the primary cause of TB and it is the most prominent members of the MTBC. It is an obligate human pathogen and are the leading causes of death all over the globe [3]. The size of the *Mycobacterium is* less than 5µ and the generation time of this bacterium is 18-24 hours [4]. They are obligate aerobes. It resists decolonization dyes like Sulphuric acid and acid alcohol because its cell wall contains mycolic acid. Therefore, this bacillus is also termed as "Acid fast bacilli" [5].

More than one third population of the world's has been infected with TB caused by MTBC, arising new infections at the speed of one per second. Annually, *M.tuberculosis* infected around 2 billion people all over the world. More than 9-10 million develop active TB disease and almost 2-3 million died because of this miserable disease [6]. Pakistan ranks fifth among high burden countries (HBC) and ranks 4th regarding the estimated number of multidrug resistance tuberculosis (MDR-TB) cases worldwide. The estimated prevalence is 341 per 100,000 populations and incidence rate is 270 per 100,000 populations respectively. The mortality rate of TB is 26 per 100,000 populations [7].

M.tuberculosis transmitted from one person to another through the air. Therefore, it is very highly transmissible disease [8]. TB cases occur predominantly in the economically most productive age group between 15 to 49 years [9]. As TB is very infectious especially in terms of pulmonary TB, therefore quick and exact diagnosis is very significant component of TB treatment and control.

2. Ziehl nelson (ZN) Staining

Conventional methods available for diagnosis of TB and the most important and easily available test is Ziehl nelson (ZN) staining for sputum [10].

2.1. Principle

This test is based on the property of acid-fastness. Mycolic acid causing acid fastness is the basic component present in the MTBC cell wall. The basic dye carbol fuchsin binds with the mycolic acid and this dye did not washed off with decolourizer i.e. strong acid or acid alcohol. Counter stain used are methylene blue which produce contrast in background.

2.2. Reagents

1% Carbol fuchsin solution, 25% H_2SO_4solution, 0.1%methylene blue solution

2.3. Methodology

Smear was prepared by means of wire loop from the samples and leave in the open air to be air dried. Smear was heat fixed and then placed for staining on staining rack. Filtered Carbol fuchsin was put on the prepared slide for atleast 5 minutes. Slide was heated underneath with flame until steam appeared. Remove the stain from the slide by tilting by means of forceps. Clean tap water was used for washing the slide. Acid solution was added to the slide for 3 minutes. Rinsed with clean tap water again. In the last step, methylene blue solution was added for 1 minute. Clean tap water was used for washing and wait for drying which is then ready for microscopic examination and appears as pinkish coulor rod in a blue background (Fig.1).

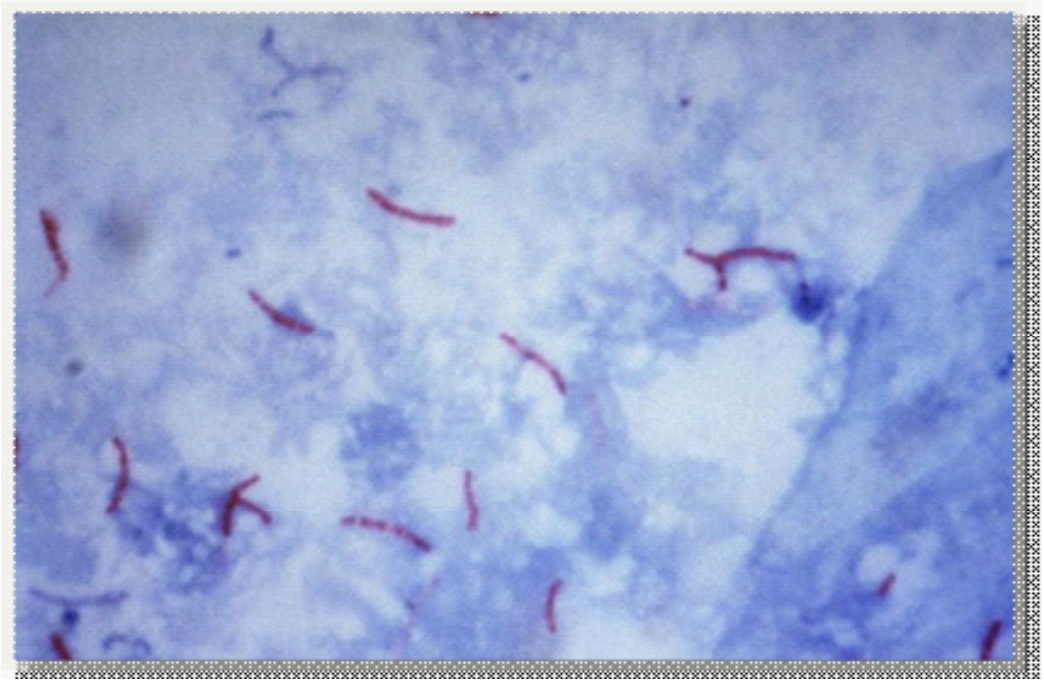

Figure 1: AFB showing in ZN staining

2.4. Reporting

Recording	Results
No AFB seenper 100 fields	Negative
1-9 AFB seen per 100 fields	exact figure reported
10–99 AFB seen per 100 fields	**1+**
1–10 AFB seen per field (count only 50 fields)	**2+**
7 10 AFB seen per field (count only 20 field)	**3+**

- Positive results was indicated by red ink and result must provide to the concerned on priority basis.
- "AFB not seen" used for a negative report while the result were not reported as "No TB" in case of negative result.

2.5. Advantages

The advantages of sputum smear microscopy are Quick, Robust, low-cost, at the door step of the majority of patients and require low level infrastructure.

2.6. Disadvantages

The disadvantages of sputum smear microscopy are need large number of bacilli is required i.e. low sensitivity up to 10^4–10^5 bacilli/ml, detects both dead and viable bacilli, does not distinguish tubercle bacilli from other mycobacteria, cannot distinguish between species of mycobacterium and no indication of drug susceptibility testing.

3. Auramine Staining

Auramine staining is another staining technique used examined by fluorescence microscopy [11].

3.1. Principle

Auramine staining technique is used for detection of AFB by microscopy. Fluorescence microscopy is used when the slide was stained with the auramine staining technique. In this staining, auramine which is the primary stain binds with mycolic acids of the cell wall. Strong acids, alcohol were used as decolourization as it does not release primary stain from the mycobacterial cell wall and bacilli seen bright yellow colour. Potassium permanganate was used as a contrast but blue ink was preferred for counter staining.

3.2. Reagents and methodology

The slide was flooded with auramine (0.1%) for 15 minutes and then washes with clean

tape water. Decolourizing solution i.e. acid-alcohol (0.5%) was flooded on the slide for 3 minutes and again washed with tape water. Slide was flooded with 0.5% potassium permanganate or 10% blue ink (Counter staining) for 1 minutes and rinse with tape water.

The fluorescence stained slide was kept in a box or folder in the dark because fluorescence fades quickly when these slides exposed to light. Therefore read as soon as possible.

The fluorescent lamp of the microscope was switched on for 5 minutes. The other ordinary lamp was off. Then rotate the nose piece so that the 20X objective of the microscope is in the light path. Checking for the strong blue light; if not, open the fluorescent light beam diaphragm. First check the positive control slide and then the test slide under the objective of the microscope. Always 40 X objectives were used for confirmation of bacilli (Fig.2).

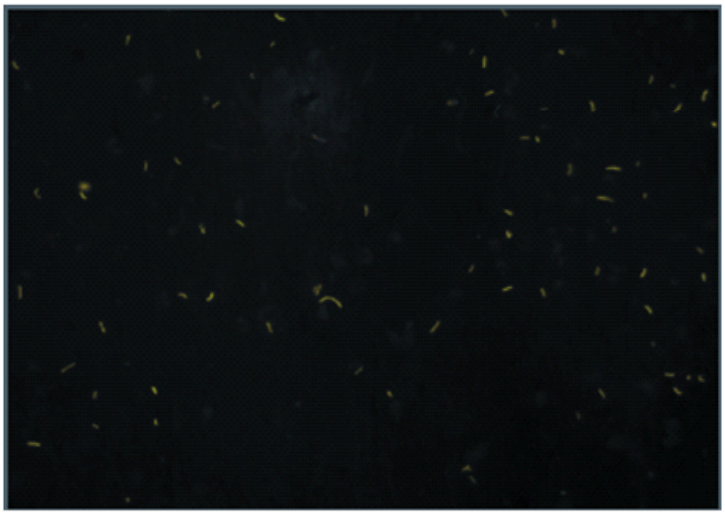

Figure 2: AFB showing in Auramine O fluorescent staining

3.3. Reporting comparison between ZN staining and Auramine staining

ZN staining	Auramine O fluorescent staining	Reporting /Grading
>10 AFB per field seen in 20 fields	>100 AFB per field seenin 20 fields	Positive, 3+
1-10 AFB per field seen in 50 fields	11-100 AFB per field seenin 50 fields	Positive, 2+
10-99 AFB seen per 100 field	1-10 AFB seen in 100 fields	Positive, 1+
1-9 AFB seen per 100 field	1-3 AFB seen in 100 fields	doubtful positive /repeat
No AFB seen in 100 fields	No AFB seen in 100 fields	Negative

3.4. Advantages

Fluorescence microscopy is better and more rapid than that of the ZN staining microscopy and it is very useful for huge work load setting. It is also more delicate for paucibacillary specimens, as it requires less effort for examination of maximum fields.

3.5. Disadvantages

However, a stable power supply is required, greater expertise of the lab technician in reading and even microscope adjustment. Moreover, a regular supply of the bulbs is also a factor in maintaining this microscopy which is so costly and short lived. Cheaper microscope using halogen lamps have less inflexible requirements.

4. Lowenstein Jenson (LJ) Media

Lowenstein Jenson (LJ) media is use for the culturing of *M.tuberculosis*. There are three main ingredients in the preparation of LJ media i.e. Salt solutions, malachite green and egg homogenate. For the production if the solid surface of the media salt solutions and egg homogenate were mixed. Malachite green was added which would help to prevent the growth of contaminants**. Stone brink media** used for the growth of *M.bovis* have the same composition as LJ medium, only pyruvate is added instead of glycerol. Pyruvate enhanced the growth of *M.bovis*.

4.1. Preparation

Reagents used in the preparation of LJ and stone brink media are salt solution, 2% malachite green solution, Hen's egg.

4.2. Methodology

Cleaned the work area with disinfectant and washed the eggs. The eggs were dipped in 70% ethanol for 15 minutes and leave them to dry. Every egg was broken separately into a sterile flask to check its quality. The eggs were transferred into another sterile beaker and mixed well by means of a stirrer. To obtain the desired volume sufficient eggs were added. Then salt solution and malachite green solution were added. Mixed gently all three solutions to avoid bubbles and attain homogeneity. Filtered through sterile cotton gauze and transferred 6 to 8 ml volumes into sterile McCartney bottles. Kept all the tubes in the inspissator at a position to form the proper slopes and coagulated at 80°C for 45 minutes. After inspissation, remove the tubes and then allowed to cool which was ready for use.

4.3. Culture

M.tuberculosis identification through culture techniques is presently the benchmark for diagnosis [12]. Culture examination is better as compared with microscopy because upto 20-50% chances of bacilli increases. Culture can detect up to 10 bacilli per ml of the sample. The time required in culture diagnosis and with the increase rate of negative results in paucibacillary specimens are the main restrictions. There are several methods for attaining early growth of *M.tuberculosis* have been developed during the last two decades [13]. The most important

method of these is decontamination of the specimens using N-acetyl L-cysteine-Sodium hydroxide (NALC–NaOH) method [14-15].

4.4. Reagents

The reagents used in the NALC–NaOH method for processing the sputum samples are the following.

4% NaOH solution, 2.9% trisodium citrate solution, NALC powder. NACL was prepared on daily basis. Took 0.25 g of NALC powder and add it in 50 ml Tri-sodium–NaOH solution.

While for the preparation of Phosphate buffer having pH 6.8,took Na_2HPO_4 and KH_2PO_4in a combination that the final pH of the buffer was 6.8.

4.5. Methodology

NALC–NaOH concentration method is the best culture process method for sputum samples decontaminated [14-15]. Sodium hydroxide acts as a decontaminating agent while NALC function as a mucolytic agent. Heavy metal ions attached by sodium citrate which may be present in the samples because they attached to NALC and NALC was non-functional by the presence of these ions. About 5 ml samples but not less than 2ml along with equal volume of the NALC–NaOH solution was transferred to the falcon tube and tightened the cap of the tube. Vortexed for 20 seconds and incubate for 15 minutes for the sample to decontaminate at room temperature. After incubation, filled the tube with phosphate buffer upto 50ml and then vortexed. Centrifuged the tubes at 3000 rpm for 15 minutes. Remove the supernatant carefully from the tube by means of a funnel into a discordant which containing 5% phenol. The sediment was resuspended by adding 0.3ml of phosphate buffer.

The sediment was inoculated on two slopes of Lowenstein Jenson (LJ) media and also with liquid media 7H9 broth using MGIT (Mycobacteria growth indicator tube) system.

The LJ media was transferred to incubator and wait for up to eight weeks at 37°C for the appearance of any visible colonies i.e. typically rough, tough and buff in colour by continuously reading for atleast one time every week (Fig.3). While in the MGIT system if the tube is contaminated, negative, positive then it detect automatically (Fig.4). The contaminated cultures in either media were discarded in the first week of incubation. In case of solid culture, if there was no growth observed after 8 weeks incubation declared as negative and after 6 weeks incubation in case of MGIT system.

4.6. Advantages

Culture detects up to 10 bacilli per ml of the sample depending on the technique used, upto 30-50% case detection rate improves over microscopy, definite and excellent diagnosis of extra pulmonary tuberculosis, MTBC species detection, allows drug susceptibility testing, allows drug resistance surveys and epidemiological studies and confirms TB in HIV+ patients.

Figure 3: Colonies of *Mycobacterium tuberculosis* on LJ media are typically rough, tough and buff in colour.

4.7. Disadvantages

Slow growth of *M. tuberculosis*, high cost, delays results, more sensitive to technical deficiencies, greater need for infrastructure i.e. qualified staff, equipment.

5. Drug Susceptibility Testing

5.1. Principle

Drug susceptibility testing using the proportion method [16] for *M. tuberculosis* along with the members of MTBC i.e. *M.africanum* and *M.bovis* against anti-TB drugs on LJ medium is also very important method. This technique is a standard method against which other methods judged. In this technique, high concentrations of viable infectious bacilli are under test, the minimum quantity of which is 109 bacilli/ml so great expertise and personal protection is very important and mandatory. Therefore, this method must be carried out only in bio safety level 3 labs which is strongly recommended by WHO and it is only access to authorized personal with maximum restriction.

As the name indicates, this method measures the proportion of resistant bacilli present in a sample which is totally different from the concept of resistance in routine clinical microbiology. The strain should be declared as susceptible when it is below a certain proportion and resistant when above that specific proportion. This proportion of the drug is known as critical proportion. The lowermost concentration of the anti TB drug indicates resistance of clinical

importance in the medium at which the growth of acid fast bacilli appears is termed as critical proportion. Growth control is also used as a control to inhibit unwanted growth on the culture medium but it is without test drug to check it with medium containing the critical concentration of the drug.

Scraped and pick up portions from all colonies of the culture medium with a sterilized loop.

5.2. Methodology

Transferred all the colonies to a screw capped glass tube containing glass beads having diameter of 3mm approximately. Gently shake the tube. 2 drops of sterile saline or distilled water was added and vortex. Wait for 15-30 minutes so that larger aggregates of bacteria were settled down. Aseptically transferred the supernatant to another tube and turbidity must match with McFarland turbidity standard No.1. If it is too turbid then sterile distilled water was added. If it is insufficiently turbid then do not add more cells because mycobacterial cells are very hard to homogenize. Let the suspension to settle down and discard some of the upper portion to concentrate cells. The turbidity of the suspension was adjusted by adding distilled water.

A strain is declared as resistant when the drug containing medium contains colonies more than that of growth control with the 1% in oculum and it is sensitive when the colonies are less than that of growth control. A strain of *M.tuberculosis* $H_{37}Rv$ is used as control. $H_{37}Rv$ is the strain which is sensitive to all first line drug of TB.

6. Nitrate Reduction Assay

Nitrate Reduction Assay is a type of biochemical test used in the biochemical identification of MTBC species. The nitrate reduction test is also used for the Drug Susceptibility Testing of MTBC. It is based on the colour change in which nitrate is converted to nitrite result in the production of colour change. The presence of this colour change can be detected by means of specific reagents. Nitrate reduction assay uses this colour change actually produce by nitrite as indication of growth in the drug susceptibility test [17].

6.1. Principle

M.tuberculosis is the strong nitrate reduction producer and reduce nitrate to nitrite measured by colorimetric method. *M.bovis* showed a negative test or a weak producer. Isolated colonies and pure cultures must be used for this test to avoid false positive test [18].

6.2. Requirements

Sodium nitrate substrates in buffer having pH 7.0, hydrochloric acid solution, 0.2%, sulfanilamide solution, 0.1% N-naphthylethylene diamine solution are the reagents used in the

nitrate reduction test.

6.3. Methodology

Took 0.2ml sterile distilled water in a tube and add pure colonies from LJ media and stone brink media to the tube. Then 2ml $NaNO_3$ solution was added and incubated at 37°C in water bath for 2 hours. After incubation, one drop of hydrochloric acid solution, 2 drops of 0.2% sulfanilamide solution and two drops of (0.1%) N-naphthylethylene diamine solution was added to the tubes. The change in colour from pink to red was noted as nitrite producer.

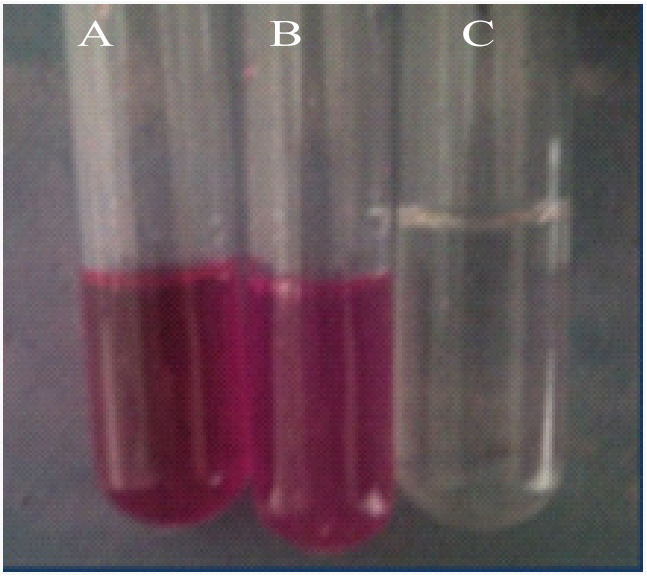

Figure 8: Nitrate reduction by *M.tuberculosis* (A) Positive, (B) control and (C) Negative.

7. Niacin Test

7.1. Principle

Niacin test is the biochemical test that identifies the accumulation of niacin in culture media and is used as for the detection of *M.tuberculosis*.

Nicotinic acid present in the niacin was detected in the niacin test and it is one of the important factors in the identification of *M.tuberculosis* in the culture media because almost all the strains of *M.tuberculosi*s excrete a large amount of niacin (nicotinic acid). Niacin test must be only carried out on pure culture of *M.tuberculosis* otherwise it produces false results. The best results obtained from 22 to 28 days old culture on LJ media having more than 50 colonies. This test must be processed in a BSC as dealing with the pure cultures.

7.2. Methodology

Took colonies from the culture and transfer to a sterile tube. 1 ml of sterile distilled water was added to the culture and tightens the caps of the tube. The tubes were placed horizontally so the fluid covers the entire surface of the medium with the fluid. Place the tube on

the room temperature for 30 minutes. Upright the slants and wait for 5 minutes so the fluid was drain to the bottom. 0.5 ml of fluid was transferred to the clean screw cap tube. The strip was inserted to the fluid having the identification mark at the top. The tube was closed immediately. Wait for 15 to 20 minutes at room temperature and agitating it occasionally. Observe for yellow colouration of the liquid in the bottom of the tube indicate a positive test against a white background.

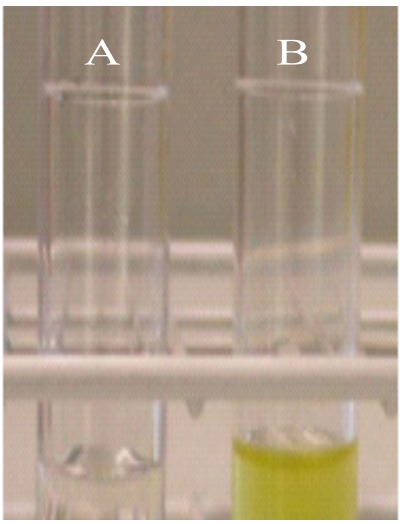

Figure 9: Niacin test by *M.tuberculosis* (A) Negative and (B) Positive.

8. Catalase Test

8.1. Principle

Catalase test is useful in the identification of *M.tuberculosis* because it detects the catalase activity from their colonies. This test was performed only in the Bio safety level 3 lab as recommended by the WHO because live and infectious bacteria are in used. The heat stability of the catalase activity was measured at 68 °C and it is one of the key factors in the identification of *M.tuberculosis*. Pure cultures of the tubercle bacilli having minimum of 14 days old was used, otherwise it produces false results.

Catalase breaks down hydrogen peroxide resulting in the production of water and oxygen. This oxygen was appearing as in the form of oxygen bubbles in the liquid suspension. These bubbles indicate the presence of all mycobacteria except is oniazid resistant bacilli and were reported as positive test.

8.2. Requirements

Phosphate buffer (pH 7.0), containing two reagents i.e. Reagent 1 consists of KH_2PO_4 (9.07 g) dissolve in 1000 ml distilled water and Reagent 2 consists of $Na_2HPO_4.12H_2O$ (23.68 g) dissolve in 1000 ml distilled water. 38.9 ml of reagent 1 was mixed with 61.1 ml of reagent 2 and check pH with a pH meter. Hydrogen peroxide (H_2O_2), Tween 80 solution, 10 ml Tween 80 was mixed with 90 ml distilled water and autoclave at 121°C for 10 minutes. It is used for

up to 3 months when store in the refrigerator. 30% hydrogen peroxide and 10% Tween 80 was mixed in equal volume. This suspension is called as substrate. It is very important to prepare a freshly prepared substrate to reduce the chances of false result.

8.3. Methodology

0.5 ml of phosphate buffer was dispensed into screw cap test tubes. Scraped several bacterial colonies from LJ media and emulsify in the buffer. Place one tube was placed in the water bath at 68 °C for 20 minutes and the other tube at room temperature. The heated tube was allowed to cool to room temperature. Then 0.5 ml of freshly prepared Tween 80 peroxide substrate was added to each tube and loose the cap of the tube. Note the bubbles formation. Care must be taken that don't shake the tube because tween 80 alone forms bubbles on shaking producing false positive results.

8.4. Results

If bubbles appear in the unheated tube and not in the heated tube, the result was interpreted as test strain belongs to the MTBC.

If bubbles appear in both tubes i.e. heated and unheated then the result was reported as this strain produces heat stable catalase and is unlikely to be a member of the MTBC.

If no bubbles appear in both tubes, then the test is reported as the test strain is catalase negative.

M.terra which is a saprophytic strain used as negative control.

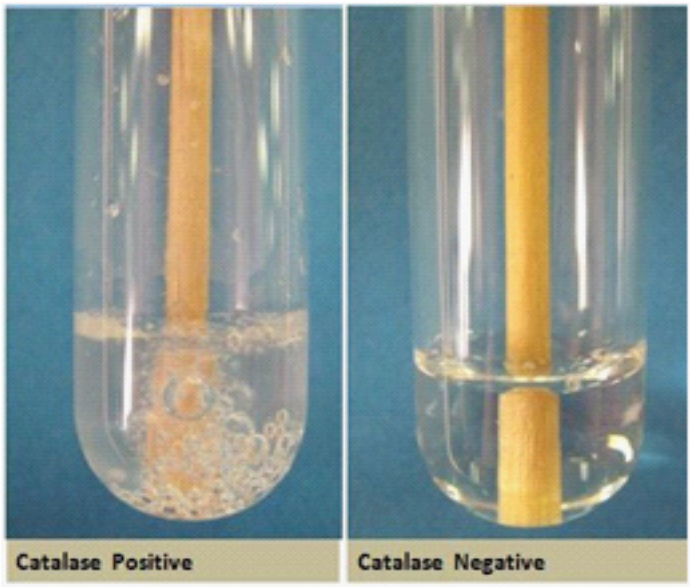

Figure 10: Catalase test

9. Mycobacteria Growth Indicator Tube (MGIT)

MGIT is the fully automatic system for detection of *M.tuberculosis* and drug susceptibility testing of MTBC. MGIT System was compared with LJ media for detection and isolation of mycobacteriaby many researchers [19-20]. Similarly, drug susceptibility testing by MGIT has been thoroughly studied [19].

9.1. Principle

It consists of liquid medium embedded with oxygen quenched fluorescent material containing silicone at the bottom of the tube. The free O_2 in the tube is utilized during bacterial growth and is replaced by CO_2. With exhaustion of O_2, the fluorochrome is no longer inhibited resulting in fluorescence at the bottom of the tube that could be detected by MGIT instrument. The intensity of fluorescence is directly proportional to the extent of O_2 reduction and rate of bacterial growth.

9.2. Methodology

This method has been developed by Becton and Dickinson. The growth is detected in the tube by a non-radioactive detection system using fluorochrome [21]. MGIT system produce improved yield and much faster growth of mycobacteria as compared to LJ media by using liquid broth medium also known as modified Middle brook 7H9 broth base. The volume of this medium is sterilized terminally by autoclaving. This media is ready for the growth of mycobacteria after adding MGIT OADC (Oleic acid, Albumin, Dextrose and Catalase) or MGIT 960 Growth Supplement. This growth supplement is vital component for growth of almost all mycobacteria, especially MTBC. Contamination is supressed by the addition of the MGIT PANTA. PANTA consists of Polymyxin B, Amphotericin B, Nalidixic Acid, Trimethoprim and Azlocillin.

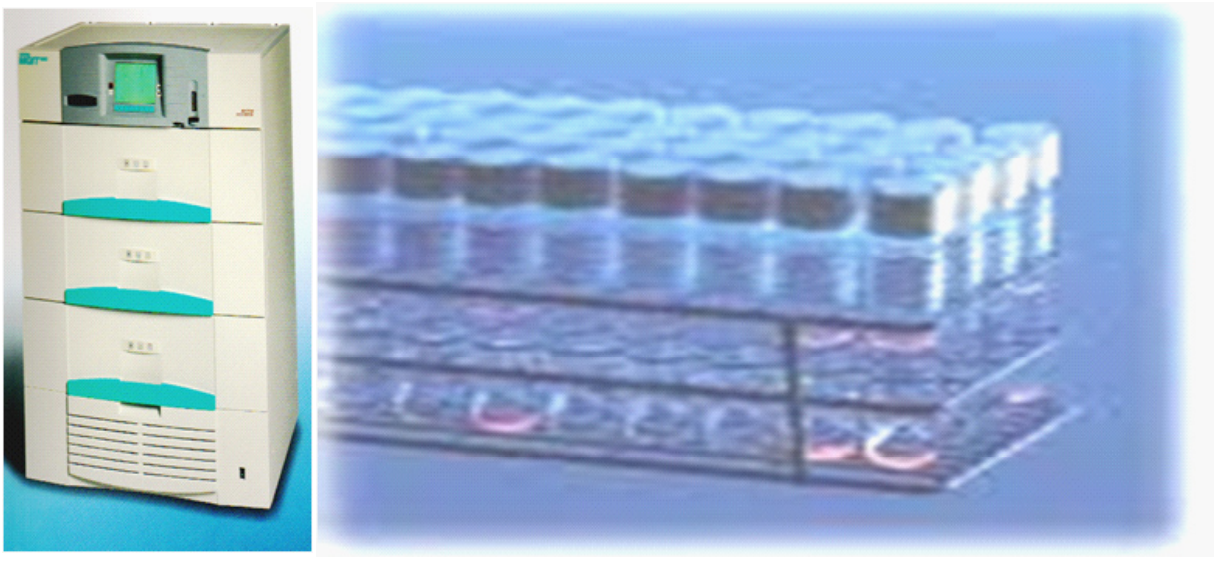

Figure 4: MGIT 960 Automated System with fluorescence seen in the positive tubes

10. Drug Susceptibility Testing on MGIT

10.1. Principle

Same principle is applied for the drug susceptibility testing on MGIT system. The test culture that required drug susceptibility testing is inoculated into two MGIT tubes. To one MGIT tube added a specific amount of test drug and compared it with other tube having no drug. Fluorescence in the MGIT tube is suppressed in case when the test drug is active against the mycobacteria and it inhibits the growth. While the fluorescence increased after the growth control will grow uninhibited. This growth is then monitored by the MGIT instrument as susceptible or resistant.

10.2. Methodology

The contaminated or mixed cultures may be concluded as positive by using liquid media. When the modified Middlebrook 7H9 broth base was declared positive by the instrument then a smear is prepared from the bottom of the tube containing the culture. Blood agar and chocolate agar plate can be used for the purity of a positive liquid culture and for the identification of possible mixed mycobacterial cultures as these are difficult to detect in the liquid media.

10.3. Advantage

MGIT increases the case detection by 10% over solid media are the main advantage of this technique.

10.4. Disadvantage

The limitation is more prone to contamination of this technology.

11. Microscopic Observation Drug Susceptibility Assay (MODS)

11.1. Principle

The decontaminated processed sputum specimens inoculated in Middlebrook 7H9 medium were introduced in 24 well plates for the presence of microcolonies by an inverted light microscope as this is much earlier than visible colonies appearance on the solid media. The isoniazid and rifampicin were also detected for the rapid identification of multidrug resistant (MDR-TB) using the same 24 well plates.

MODS detect and identify *M.tuberculosis* along with isoniazid and rifampicin susceptibility by liquid culture directly from the samples [22].

There are two main advantages of this techniques regarding growth of *M.tuberculosis:*

(1) Liquid media produces faster growth of *M. tuberculosis* as compared to solid LJ media.

(2) *M.tuberculosis* typical cording characteristic appearance in liquid media.

11.2. Reagents

The reagents used in the process are the decontamination reagents for the sputum process are PBS (PH 6.8), NaOH (4%) and Na-Citrate (2.9%), NALC (0.5%) which is NALC-NaOH solution.

Middlebrook 7H9 broth base containing Glycerol and Casitone (4.5 ml aliquotes in glass tubes), OADC; supplement (oleic acid, albumin, dextrose, catalase) commercially prepared, PANTA which is Lyophilized antibiotic supplement i.e. Polymyxin-B, Amphotericin-B, Nalidixic acid, Trimethoprim, Azlocillin) and antibiotic isoniazid & Rifampicin stock solutions (8mg/ml). Aliquotes of 20ul were prepared and store at -20^{o}C for 6 months.

11.3. Methodology

Sputum samples having volume of not less than 2ml were decontaminated using NALC–NaOH concentration method [19-20]. Sputum samples along with equal volume of the NALC–NaOH solution were added to the tube. Tighten the cap of the tube and vortexed. Leave the tube for 15 minutes at room temperature to decontaminate the samples. Phosphate buffer was added upto 50ml of the tube and again vortexed. The tubes were centrifuged at 3000 rpm for 15 minutes. The supernatant was carefully removed through a funnel into a discordant containing 5% phenol. The pellet was resuspended by adding 0.3ml of phosphate buffer.

OADC was added to 7H9 medium i.e. 7H9-OADC in a clean room or Biological Safety Cabinet (BSC). PANTA was reconstituted and add to tubes with 7H9-OADC. The tube now consists of 7H9-OADC-PANTA. The resuspended pellet was added to the 7H9-OADC-PANTA. Antibiotic working solutions was prepared in a 24 well plate. The antibiotic working solutions was added to plated samples.

The plate was closed with lids and sealed each in a ziplock bag. Placed in incubator at 37°C. Read the plates on day 5 of incubation by means of inverted microscope. Plates are examined within the transparent and sealed zip lock bags and not opened.

After 5 to 9 days of incubation, mycobacterium growths are like small curved commas shaped and spirals. The colony formation of mycobacterium usually turned to cords and then later develops to irregular tangled. The results are declared as positive, when two or more than two colonies are detected in each well i.e. ≥2 CFU.

11.4. Reporting

When there is no colony detected after day 5 incubation, then the drug free well are further incubated and continue reading daily or on the alternate day depending upon the workload until two or more colonies are detected (≥2 CFU). When the drug free well was positive then observe the growth of the wells containing isoniazid and rifampicin on the same day. While if no growth detected till day 15 then continue the incubation to 18 and atlast to day 21. At day 21, if the result is still negative the final report was reported as negative.

In case of only 1 CFU detected in drug or drug free well then report the result as "indeterminate". The drug well should only read when the drug free well was positive and not read when the drug free well was negative or indeterminate. In case of bacterial or fungal contamination appearance in the well then the sample should be re-decontaminated, or a fresh sample was requested and the result was reported as un-interpretable.

11.5. Interpretation

The Resistance of this method is defined as when two or more than two colonies are detected in the drug containing wells on the same day that both drug-free wells are positive.

All positive cultures are detected within 21 days and >98% within two weeks thus a negative MODS culture at three weeks is a confident rule-out.

11.6. Advantage and Disadvantage

This technique is very simple, the liquid media sensitivity has great advantage over solid media for TB detection, characteristic growth of *M. tuberculosis* specificity, short duration for the detection of drug susceptibility testing and low cost of reagents are the major advantages.

MODS are recommended only for smear positive samples and not for smear negative specimens are the only limitation.

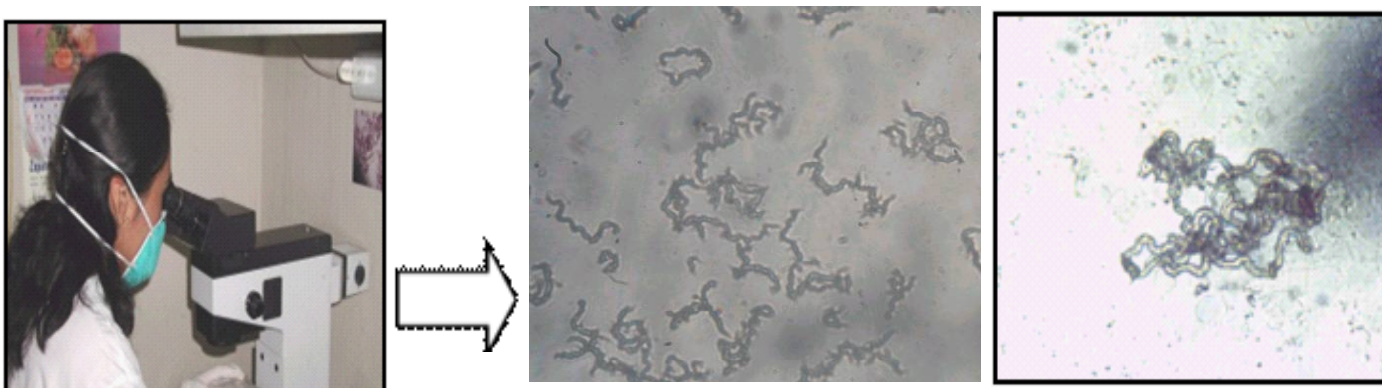

Figure 5: MODS: Inverted Microscope showing colony morphology of AFB

12. Line Probe Assays (LPA)

12.1. Principle

LPA was first of all introduced in 2004, identifies MTBC and simultaneously identifies mutations in the *rpoB* gene as well as mutations in the *katG* gene for high-level isoniazid resistance.

LPA is currently the useful and faster available technology for the rapid identification of Rifampicin and isoniazid resistance, but the only disadvantage of this technology is that it is only used in smear-positive specimens. It is technically complex procedure, extensive infrastructure required because the chances of cross-contamination. It is only suitable and available at reference laboratory [23].

12.2. Methodology

The main steps involved in the LPA are *M.tuberculosis* DNA was extracted from culture isolates or directly from smear positive specimens. Next, DNA was amplified by polymerase chain reaction (PCR) cycles of the resistance determining region of the gene using biotinylated primers was performed. After amplification was performed, a strip containing specific oligonucleotide probes was used for hybridization of the labeled the PCR products. Colorimetric method was used for the identification of these captured labeled hybrids. These labeled hybrids shows the identification of *M.tuberculosis* along with the detection of wild-type and mutation probes for is oniazid and rifampicin resistance [24].

If one of the target regions shows mutations, the amplicons will not hybridize with the relevant probe. Mutations are detected by the presence of mutation probe and lack of wild-type probes. A coloured band was detected on the strip after post hybridization reaction at the site of probe binding which is clearly observed by human eye [25]. The different types of *Mycobacterium* species identified by line probe assay (Fig. 6).

12.3. Advantage

Smear negative specimens are not recommended on the LPA. This technology is only applied to and validated in direct smear positive samples and culture isolates of *M.tuberculosis* complex which is isolated from smear negative and smear positive samples.

12.4. Disadvantage

Three rooms are required for the performance of LPA in Laboratory i.e. DNA extraction room, pre-amplification room and post-amplification room. In these rooms unidirectional flow of work, only authorized personal allowed and cleaning procedure must be established to reduce amplicons contamination which leads to false positive results.

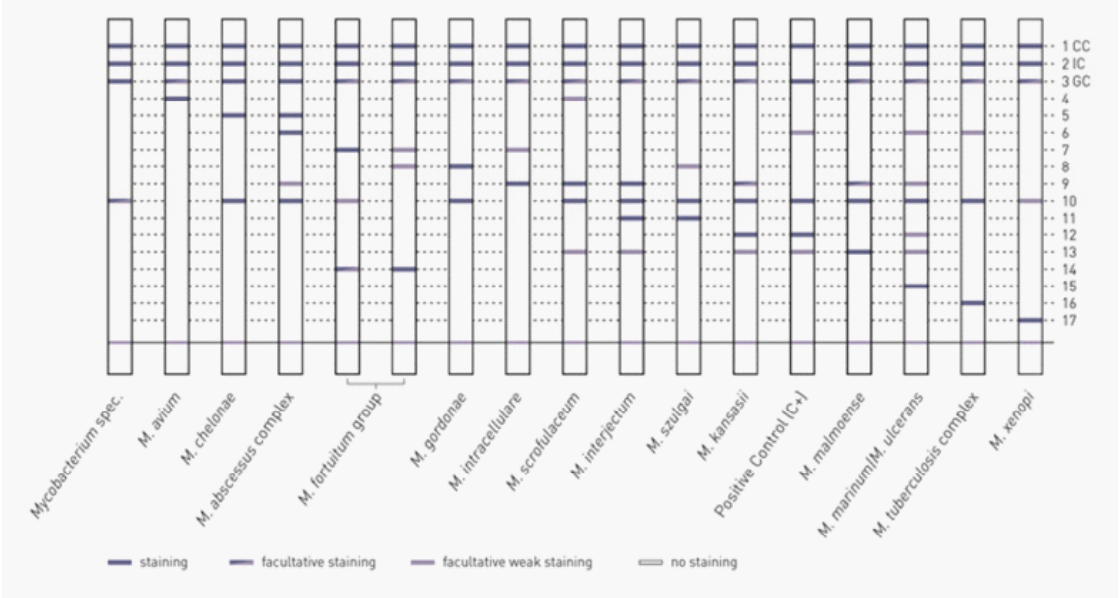

Figure 6: Line Probe Assay showing identification of different organisms

13. GeneXpert

13.1. Principle

GeneXpert was development in 2009 and it is considered a vital revolution in the competition against TB. A simple molecular test was introduced which is robust and performed outside the laboratory settings for the first time. GeneXpert MTB/RIF assay identified not only *M.tuberculosis* but also rifampicin resistance. The resistance conferring mutations was detected by using three specific primers and five unique molecular probes to confirm a high degree of specificity.

13.2. Methodology

The MTB/RIF assay required less than 2 hours for the results performed directly from specimens. The GeneXpert System automates and integrates DNA extraction/ sample preparation, DNA amplification and detection of the target DNA by using real time polymerase Chain Reaction (PCR). The patient sample was diluted with buffer which was provided with the kits and then incubated for 15 minutes at room temperature. The sample was completely liquefied by mixing to and fro motion after every 5 minutes during incubation. The liquefied sample was transferred to cartridge and gives it to the GeneXpert system. The GeneXpert system gives both summarized and detailed test results in tabular and graphic formats in less than 2 hours.

GeneXpert MTB/RIF system consists of GeneXpert instrument, scanner with barcode reading, computer and GeneXpert software for tests running on different samples and then viewing the results (Fig.7). The test was performed on disposable single use GeneXpert cartridges which hold the PCR reagents and PCR reactions were performed. These cartridges are closed systems so the chances of cross contamination were eliminated between samples. This cartridge include two controls a sample processing control (SPC) which control the processing

of the target bacteria and also monitor the detection of inhibitor in the PCR reaction. The Probe Check Control (PCC) used for the verification of reagent rehydration, proper filling of the PCR tube in the cartridge, probe integrity and dye stability.

Figure 7: GeneXpert- MTB RIF Test showing cartridge and instruments

13.3 Advantage

GeneXpert gives very fast results because an individual can know the result within the same day. It not only diagnoses TB but also detect if the TB of the person is sensitive or resistant to rifampicin. By knowing the status about rifampicin then it is very easily define about the treatment regimen combination and TB can be treated effectively. Up to about 98% Gene Xpert is also more sensitive as compared to other TB tests so it is a very good instrument. It is also detect TB especially in AIDS patient and in people with compromised immune systems because it is often seen that the test is negative for TB even though they are sick with this disease.

13.4. Disadvantages

Gene Xpert is much more costly in comparison with sputum-smear microscopy tests. GeneXpert also needs calibration at least once a year which is also very expensive procedure. Test takes upto two hours without the interruption of electricity for a single second. A computer is also needed for this important instrument. Therefore, it is very essentials that all laboratory setups and hospitals need proper and reliable arrangement and security measures for this important instrument.

14. Future Research Needs

Additional studies to assess the performance of the different tests under program conditions should be conducted. Further research is needed regarding use of the tests for the identification of *Mycobacterium tuberculosis* in multiple clinical circumstances. Studies of test performance should assess specificity, sensitivity, reproducibility, and association of test results

with risk for infection and risk for progressing to TB disease. Comparisons among different basic, culture and biochemical tests are encouraged.

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Diagnosis and Management of Tuberculosis

Chapter 6

Tuberculosis: New Laboratory Diagnostic Methods and Treatment

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1. Introduction

Tuberculosis (TB) still remains one of the world's deadliest communicable diseases. The World Health Organization (WHO) estimated that there were 10.4 million new TB cases worldwide and 1.4 million TB deaths in 2015. People living with HIV accounted for 1.2 million (11%) of all new TB cases [1]. Because of the slow growth rate of the causative pathogen *Mycobacterium tuberculosis*, isolation, identification, and drug susceptibility testing (DST) of this organism can take several weeks or longer. In recent years, many new laboratory diagnostic methods have been developed for direct detection and susceptibility testing of TB. A good understanding of their effectiveness and limitations is important to improve TB diagnosis.

M. tuberculosis is a tough and resilient microorganism that is well adapted to prolonged residence in its human host. Shielded by a waxen cell wall that protects against lethal enzymes and other deadly products elaborated by the body's antibacterial defenses, tubercle bacilli are also sheltered against foreign chemicals such as gold, arsenic, mercury, calcium, iodine, quinine, creosote, turpentine, cod liver oil, and chaulmoogra oil, to mention just some of the many

"therapeutic" substances of historical interest that had been tried in a fruitless effort to arrest or reverse the progress of consumption. The goals of tuberculosis treatment are to ensure cure without relapse, to prevent death, to stop transmission, and to prevent the emergence of drug resistance. *M. tuberculosis* can remain dormant for long periods. The number of tubercle bacilli varies widely with the type of lesion, and the larger the bacterial population, the higher the probability that naturally resistant mutants are present even before treatment is started. Even up to now, treatment of tuberculosis is still a challenge work.

2. New Laboratory Diagnostic Methods of Tuberculosis

2.1. Xpert MTB/RIF assay

Xpert MTB/RIF is a new self-contained and cartridge-based assay based on the GeneXpert multi-disease testing platform, that dramatically simplifies molecular testing by fully integrating and automating the three processes required for real-time PCR-based molecular testing: sputum processing, DNA extraction and amplification, TB and drug-resistant TB diagnosis. It has similar sensitivity to culture, targets *M. tuberculosis* specifically and enables simultaneous detection of rifampicin resistance via the *rpo*B gene [2]. Xpert MTB/RIF is a simple, rapid, automated molecular assay. The 1-step external sample preparation is extremely simple. The closed system ensures that there is no risk of contamination and no requirement for bio-safety facilities. Test results can be obtained in just 90 minutes.

After completion of development in 2008, the diagnostic accuracy of the Xpert MTB/RIF assay for pulmonary TB, done by the Foundation for Innovative and New Diagnostics (FIND), were assessed in 1730 patients with suspected drug-sensitive or multidrug-resistant pulmonary TB in Peru, Azerbaijan, South Africa and India. The result showed that among culture-positive patients, a single, direct Xpert MTB/RIF test identified 98.2% of patients with smear-positive TB and 72.5% with smear-negative TB. The test was specific in 99.2% of patients without TB. Among patients with smear-negative, culture-positive TB, the addition of a second Xpert MTB/RIF test increased sensitivity by 90.2%. As compared with phenotypic drug-susceptibility testing, Xpert MTB/RIF testing correctly identified 97.6% of patients with rifampin-resistant bacteria and 98.1% with rifampin-sensitive bacteria [3].

A recent review, which included 27 unique studies (integrating nine new studies) involving 9557 participants, indicated that as an initial test replacing smear microscopy, Xpert MTB/RIF sensitivity was 89% and specificity 99%, while as an add-on test following a negative smear microscopy result, the sensitivity was 67% and specificity was 99%. For smear-positive, culture-positive TB, Xpert MTB/RIF sensitivity was 98%. For people with HIV infection, the sensitivity was 79%, and for people without HIV infection, it was 86%. In comparison with smear microscopy, Xpert MTB/RIF increased TB detection among culture-confirmed cases by 23%. For rifampicin resistance detection, Xpert MTB/RIF sensitivity was 95% and specificity

was 98% [4].

WHO had endorsed Xpert MTB/RIF technology and released a recommendation and guidance for countries to incorporate this new technology in their programs [5]. This assay was specifically recommended for use as the initial diagnostic test for suspected drug-resistant or HIV-associated pulmonary tuberculosis. By June 2012, two-thirds of countries with a high TB burden and half of countries with a high multidrug-resistant (MDR)-TB burden had incorporated the assay into their national tuberculosis programme guidelines. In September 2013, Xpert MTB/RIF was approved by FDA [6].

The main disadvantages of Xpert MTB/RIF assay are high cost and high false positive rate in areas with low prevalence of rifampicin resistance. A recently updated WHO policy has recommended that a repeated Xpert MTB/RIF assay on a fresh specimen can be useful when it detects *M. tuberculosis* with RIF resistance in the patients considered to be at low risk of MDR-TB [7].

2.2. Line probe assay (LiPA)

LiPA is a DNA strip test combined with a reverse hybridization method that allows simultaneous molecular identification of tuberculosis and the most common genetic mutations causing resistance to rifampicin and isoniazid or the 2nd line drugs. This technology can diagnose MDR-TB or extensively drug-resistant (XDR)-TB directly from smear-positive sputum samples, providing results in just five hours - an enormous improvement on the 1 to 2 months needed for conventional DST for 1st line and 2nd line drugs.

GenoType Mycobacteria Drug Resistance (MTBDR) (Hain Lifescience GbmH, Germany) is one of the most studied LiPA commercial assays. The MTBDR assay (first version introduced in 2004) identifies mutations in the *rpo*B gene as well as mutations in the *kat*G gene for high-level isoniazid resistance [8]. The GenoType MTBDR plus, the second-generation assay, can further detect mutations in the *inh*A gene that confers resistance to low-levels of isoniazid [9]. The GenoType MTBDRsl assay can determine the genetic mutations associated with resistance to fluoroquinolone, aminoglycosides (kanamycin, amikacin), cyclic peptides (capreomycin), ethambutol, and streptomycin [10]. INNO-LiPA Rif. TB assay (Innogenetics, Belgium) is another commercialized LiPA, and also has been shown to be highly sensitive and specific in the detection of RIF-resistant *M. tuberculosis* complex (MTBC) isolates [11,12]. However, it cannot evaluate the mutations leading to isoniazid resistance [13].

Evaluation studies by FIND and others indicated that the LiPA is highly accurate in detecting MDR-TB in a variety of geographical settings, and cost-effective when compared with TB culture and DST. They also demonstrated significant patient benefits, including early targeted treatment of MDR-TB and the potential interruption of transmission. A review indi-

cated that the pooled sensitivity and pooled specificity of the MTBDRplus assay were 95.9% and 98.0%, respectively, and the sensitivity of the INNO-LiPA Rif. TB assay for rifampicin resistance was 94.1% and pooled specificity was 98.8%. Both of them maintained high negative predictive value (NPV) (greater than 95%) across prevalence rates. However, at lower prevalence rates (3%), the positive predictive value (PPV) was less than 90% for both the INNO-LiPA Rif. TB assay (71.0%) and the MTBDRplus assay (59.3%). At a higher prevalence rate of 15%, the PPV was improved, 93.3% for the INNO-LiPA Rif. TB assay and 89.2% for the MTBDRplus assay [14].

The reported sensitivity for detecting ofloxacin, amikacin, and extensive drug resistance, using the GenoType MTBDRsl, was 90.7%, 100% and 92.3%, and the specificity for detection was 98.1%, 99.4% and 99.6%, respectively. GenoType MTBDRsl revealed a significant increase in diagnostic yield of 20.1% and 19.3% for ofloxacin and amikacin resistance, respectively. In addition, implementation of this test significantly reduced the turn around time by 93.3%, calculated from the date that the specimen was received at the laboratory to reporting second-line results [15].

In 2008, the WHO issued a recommendation for the use of molecular LiPA for the rapid diagnosis of MDR-TB in high TB-burden, low-income settings. In March 2012, the Expert Group of WHO recommended that the Genotype MTBDRsl assay can not be used as a replacement test for conventional phenotypic DST. However, they noted that this technology may be used as a rule-in test for XDR-TB where line probe assay capacity is available [16]. Given high assay specificity, results could be used to guide infection control measures to interrupt transmission. Low automatization, requirement of specialized laboratory and expertise including PCR are primary disadvantages of this technique.

2.3. Loop-mediated isothermal amplification (LAMP)

LAMP is a recently developed molecular method that has been successfully implemented in the detection of *M. tuberculosis* in clinical specimens [17]. LAMP has several advantages, such as rapidity, high sensitivity, ease of application and cost-effectiveness. The test amplifies target DNA at a constant temperature, meaning that it can be carried out with minimal equipment at low-level laboratories such as those in TB endemic countries. LAMP has high specificity because of the high specificity of the target sequence, and multiple independent sequences are identified by the primers.

In April 2012, WHO convened an Expert Group to review data from field evaluation and demonstration studies on the performance of the TB LAMP assay. The Expert Group agreed that LAMP technology has potential as a rapid TB diagnostic tool but that the body of evidence presented on the assay was insufficient to make a recommendation either in favor of, or against the use of TB LAMP as a replacement test for sputum smear microscopy. The Expert

Group made several recommendations for conducting further research and studies to improve the evidence base for the technology. A multicenter study in 2016 showed TB-LAMP (Eiken Chemical Co.) sensitivities among culture-positive samples were 97.2% (243/250) and 62.0% (88/142) for smear-positive and smear-negative TB, respectively, but varied widely by country and operator. Specificities ranged from 94.5% (446/472) to 98.0% (350/357) by country. A root cause analysis identified high temperatures, high humidity, and/or low reaction volumes as possible causes for false-positive results, as they may result in nonspecific amplification [18]. Gelaw et al. assessed the diagnostic performance of LAMP assay in detecting *M. tuberculosis* infection in sputum sample compared to LED fluorescent smear microscopy. They found the agreement between the two tests was very good (kappa = 0.83, P-value </=0.0001), and LAMP showed similar specificity but a slightly lower sensitivity with LED fluorescence microscopy [19]. Nliwasa et al. found the sensitivity of LAMP was similar to Xpert MTB/RIF but lower than fluorescence smear microscopy and all three tests had high specificity [20].

2.4. Interferon gamma release assay (IGRA)

IGRA is an in vitro immune test that has been introduced in recent years for the diagnosis of tuberculosis infection. IGRA is based on the detection of a T-cell immune response towards *M. tuberculosis* complex specific antigens (culture filtrate protein (CFP)-10, early secretory antigenic target (ESAT)-6 and/or TB7.7). To date, there are two most commonly used commercial forms of IGRA: the enzyme-linked immunospot (ELISPOT)-based T-SPOT®.TB test (Oxford, UK), and the enzyme-linked immunosorbent assay (ELISA)-based QuantiFERON®-TB Gold in-tube assay (QFT-GIT) and its predecessor QuantiFERON®-TB Gold (QFT-G) test (Cellestis, Australia).

Latent *M. tuberculosis* infection (LTBI). IGRA may have a relative advantage over the tuberculin skin test (TST) in detecting LTBI and allow the exclusion of *M. tuberculosis* infection with higher reliability [21]. Both IGRA and TST have low sensitivity in a variety of immunocompromised populations [22]. IGRA were more sensitive than TST for diagnosis of *M. tuberculosis* infection in HIV-infected patients [23]. Both TST and current IGRAs primarily detecting a CD4 T-cell response have low predictive value for progression from infection to active TB [22]. However, a new generation assay, the QuantiFERON-TB Plus (QFT-Plus, Qiagen, Hilden, Germany), has been developed to stimulate IFN-γ production by both CD4 and CD8 T-cells. First results indicate that the CD8 T-cell response may be able to identify people at greater risk of progression to active TB [24].

Active TB. In blood and extra-sanguineous fluids, the pooled sensitivity for the diagnosis of active TB was 80% and 48% for QFT-GIT and 81% and 88% (confirmed and unconfirmed cases) for T-SPOT, and the pooled specificity was 79% and 82% for QFT-GIT and 59% and 82% for T-SPOT, respectively [25]. Among HIV-infected adults with active pulmonary

tuberculosis in low- and middle-income countries, pooled sensitivity were 76% for T-SPOT and 60% for QFT-GIT, and pooled specificity were 52% for T-SPOT and 50% for QFT-GIT [26].

Extrapulmonary TB (EPTB). A meta-analysis showed pooled sensitivity for the diagnosis of EPTB was 72% for QFT-G or GIT and 90% for T-SPOT, and pooled specificity for EPTB was 82% (QFT-G or GIT) and 68% (T-SPOT) [27]. A meta-analysis on pleural tuberculosis showed the pooled sensitivity and specificity for the blood assays were 77% and 71%, and for the pleural fluid assays were 72% and 78%. There was considerable heterogeneity. They concluded that commercial IGRAs, performed either on whole-blood or pleural fluid, have poor diagnostic accuracy in patients suspected to have tuberculous pleural effusion (TPE) [28]. A meta-analysis on tuberculous meningitis showed the moderate diagnostic accuracy of blood and cerebrospinal fluid (CSF) IGRA. The overall sensitivities for blood and CSF IGRAs were 78% and 77%, and the specificities were 61% and 88%, respectively [29].

Pediatric TB. Two meta-analysis studies showed the sensitivities of two IGRAs and TST in active pediatric tuberculosis were similar (70% for ELISA, 62% for ELISPOT and 71% for TST). The pooled specificity was 100% for ELISA and 90% for ELISPOT, but was much lower for TST (56% in all included studies and 49% in children with bacillus Calmette-Guerin vaccination). So IGRAs performance in children showed no better sensitivity than TST, but higher specificity [30,31].

In conclusion, current IGRAs are costly and require fairly sophisticated laboratory infrastructure and technical expertise. Their performance differs in high versus low TB and HIV incidence settings, with relatively lower sensitivity in high-burden settings. IGRAs (like the TST) cannot distinguish LTBI from active TB, and should not be used for the diagnosis of active TB disease in high-burden settings due to a high background prevalence of LTBI [32].

2.5. Urine lipoarabinomannan (LAM) test

Lipoarabinomannan is an important 17.5-kD heat-stable glycolipid found in the cell wall of *M. tuberculosis*, accounting for up to 15% of the total bacterial weight [33]. Detection of urine LAM has several advantages compared with currently used diagnostics. It is non-invasive, simple to collect, process and store, and is attractive in people with no sputum. And it has been used to develop commercially available enzyme-linked immunosorbent assays, such as Determine TB-LAM (Alere, Waltham, MA, USA) [34].

A meta-analysis in 2011 regarding use of urine LAM assays for diagnosing active TB showed sensitivity ranged from 13% to 93%, while specificity ranged from 87% to 99% in microbiologically confirmed cases, sensitivity ranged from 8% to 80%, while specificity ranged from 88% to 99% in clinical and confirmed TB cases, sensitivity was 3-53% higher in HIV-

positive than HIV-negative subgroups, and sensitivity was highest with advanced immunosuppression [35]. It suggests urine LAM assay has suboptimal sensitivity for routine clinical use. However, Lawn et al. evaluated the diagnostic accuracy of Determine TB-LAM and found it provided results within 30 min and had highest sensitivity at low CD4 cell counts: 66.7% at <50 cells per muL, 51.7% at <100 cells per muL, and 39.0% at <200 cells per muL; specificity was greater than 98%. It provides important incremental yield when combined with sputum smear microscopy, which did not differ statistically from the sensitivities obtained by testing a single sputum sample with the Xpert MTB/RIF assay. Urine LAM test is a simple, low-cost, alternative to existing diagnostic assays for tuberculosis screening in HIV-infected patients with very low CD4 cell counts [34]. Other studies also reported similar findings [36-38].

Kroidl et al. evaluated the diagnostic performance of two urine LAM tests (MTB-LAM-ELISA assay and the Determine TB-LAM-strip assay) in children with suspected tuberculosis (TB) in a high TB/HIV-prevalence setting. They found the assays' sensitivity was higher in HIV-positive versus HIV-negative children: 70% versus 13% for MTB-LAM-ELISA and 50% versus 0% for Determine TB-LAM. In 35 children with excluded active TB, both assays showed a specificity of 97.1%. In addition, LAM excretion declined to zero during or at conclusion of antituberculous treatment in most patients, suggesting its potential as a treatment-monitoring tool [39]. Another recent study found that the urinary LAM level was higher in children with TB compared to non-TB group ($p<0.001$). Urine LAM had 83% sensitivity and 85% specificity with cut off value 0.98 mg/l using microbiological and clinical confirmation as standard reference. Because the clinical presentation is not specific, the chest X-ray interpretation has low accuracy and sputum sample is difficult to obtain in children, urine LAM test may be a rapid non-invasive alternative for paediatric TB diagnosis [40].

3. Treatment of Tuberculosis

3.1. Chemotherapy of drug-susceptible Tuberculosis

3.1.1. The prestreptomycin era

Between 1925 and 1935, Hart indicated that sanocrysin, a gold salt, was widely used in tuberculosis treatment [41]. A number of different sulphones that had activity in experimental animals were also investigated but were never widely used in treatment. Vitamin D was also explored in early work, as was nicotinamide, from which several current antituberculosis drugs, including isoniazid (INH) and ethionamide, were subsequently developed as analogs. The basis of treatment was, however, rest for the patient in sanatorium and rest for the affected portion of the lung by collapse therapy through operative procedures on the chest wall (thoracoplasty) and the injection of air into the pleural cavity (artificial pneumothorax) [42]. Pulmonary tuberculosis was reputed to have a 50% mortality, with tuberculous meningitis and miliary tuberculosis uniformly fatal.

3.1.2. Early clinical trials on Tuberculosis

After the discovery of streptomycin (SM) [43] and the proof of its antituberculosis activity in the guinea pig, small uncontrolled studies were undertaken in the United States, but the first clinical trial with a randomized intake in the history of medicine was started in1946 by the British Medical Research Council [44]. Because only a limited amount of SM was available in the United Kingdom, patients with advanced pulmonary disease could ethically be randomized to treatment with bed rest alone or bed rest plus 2 g SM daily. The results showed a substantial immediate advantage to the SM arm, but most patients developed SM-resistant strains, and the results of a 5-year follow-up indicated that they had little eventual benefit compared with the control arm. This study focused the aim of development during the next 20 years on preventing the emergence of drug resistance. In contrast to the results in pulmonary tuberculosis, a parallel study showed that SM was able to cure about 44% of patients with tuberculous meningitis [45]. Drug resistance did not emerge in these patients because the bacterial population was too small to contain resistant mutants.

The next step was to conduct a randomized controlled trial (RCT) comparing treatment of acute pulmonary tuberculosis with either SM or p-aminosalicylic acid (PAS) or with both SM and PAS [46]. The aim was to inhibit SM-resistant mutant bacilli with PAS, a very weak drug on its own. The results of this study showed that SM and PAS induced far fewer SM-resistant strains than SM alone. Isoniazid (INH), introduced in 1952, was a more potent drug than SM or PAS, probably because it can be given safely at a dose size substantially above the minimal effective dose. Between 1952 and the mid 1960s, a series of RCTs on combinations of INH, SM and PAS were performed by the British Medical Research Council, the U.S. Veterans Administration, the U.S. Public Health Service, and elsewhere. In 1955, the British Medical Research Council performed the first national drug resistance survey, which showed that almost all strains with primary resistance were resistant to only one drug [47,48]. Because of this finding, treatment with an initial three-drug phase lasting 2 to 3 months, followed by a continuation phase with two drugs was explored first in Scotland and then internationally [49]. Whereas the regimen was highly successful and was adopted as standard in the Western world, it had to be given for at least 12 months with resulting frequent failures to complete treatment. Furthermore, it was too expensive in drug costs (particularly for PAS) to be widely used in developing countries. The need to reduce costs in developing countries led to a series of RCTs in East Africa on thiacetazone (TB1) as a cheaper alternative to PAS. The possible use of INH alone was also explored [50] on drug cost grounds and because the low guinea pig virulence of highly resistant strains suggested that they might not cause progressive human disease [48, 51]. Unfortunately, this thesis proved not to be true [52], and because initial INH resistance carried a poor prognosis, the use of INH in monotherapy has been abandoned.

During the course of these and related studies, it was possible to assess the relative merits of other antituberculosis drugs by their ability to prevent the emergence of INH resistance when used in a double-drug regimen with INH. Whereas rifampin (RMP) was the most effective, SM and ethambutol were a little less effective, and PAS and TB1 much less effective. Estimation of SM and INH concentrations, and later also RMP were made in several different types of tuberculous tissue obtained at resection soon after a drug dose was given. They showed that these drugs penetrated throughout lesions and caseous matter in concentrations adequate for bacteriostatic. Thus, there were no compartments, such as thick-walled cavities, into which these drugs failed to penetrate. Indeed, if this were not true, it would be difficult for combined therapy to be effective because there would always be compartments in which only one drug would be active and would therefore create resistant strains.

From early on in the development of chemotherapy, the rationale for the slow fall in counts of viable bacilli during treatment presented a problem. The existence of persisting bacilli was recognized early and thought likely to occur when bacilli were in a stationary phase of growth or under anaerobic conditions [53,54]. However, perhaps the most important advance in the chemotherapy of tuberculosis was the series of long-term studies of experimental tuberculosis in mice performed in Walsh McDermott's Department at Cornell University on pyrazinamide (PZA) [55]. A model system was established in which treatment with drugs was given to infected mice for periods of 3 months or even longer, and counts of viable bacilli in the organs were done throughout treatment. With slight modifications, this model is still being used today. PZA, reviewed recently [56], was discovered in 1952. It is a remarkable drug that does not appear to have a genetic site of action but accumulates within the bacterial cell, where it acidifies its content and damages membranes. Unlike any other drug, as bacterial metabolism slows down PZA becomes more bactericidal.

In the murine model, therapy with standard drugs-INH, SM, PAS-produced an initial fall in viable counts, but these then leveled out and it was difficult to sterilize the organs. When PZA was added, the counts continued downwards and eventually a state was reached in which all organ cultures were negative. PZA is thus a good sterilizing drug. However, because bacilli in a nonculturable form were still present, relapses eventually occurred. Similar experiments were performed later at the Pasteur Institute, Paris, showing the high sterilizing activity of RMP. In vitro experiments showed that the reason for the high sterilizing activity of RMP probably lay in the speed with which it started to kill bacilli as they recovered from dormancy and not to a particularly rapid kill of slowly growing bacilli. A hypothesis was put forward to explain the activities of different drugs, on the basis of the presence of widely different growth rates within the bacterial population at the start of treatment.

3.1.3. Development of short-course chemotherapy

As a direct result of the two sets of experiments on the treatment of murine tuberculosis with PZA and RMP, the multicenter RCT that established short-course chemotherapy was performed in East Africa in 1970. All of the patients were in hospital throughout treatment to be sure that their prescribed treatment was actually taken. They were allocated at random 6-month regimens of (1) daily SM and INH (SH); (2) SH with the addition of RMP; (3) SH with the addition of PZA; and (4) SH with the addition of TB1, given for 12 months as a control. After completion of treatment the patients were followed up with monthly bacteriology for 24 months. The primary endpoint was the rate of relapse during follow-up and the secondary endpoint was the proportion of patients who had a positive sputum culture at 8 weeks. This was the first RCT in which these end-points were used, which are the same as those currently in operation for modern RCTs. The results show the great reduction in relapse rates in the regimens containing RMP and PZA with a slight superiority of the regimen with RMP. These are the results that led to a burst of RCTs under the auspices of British Medical Research Council in East Africa, Hong Kong, Singapore, Madras, Algeria, and Prague [57]. A few years later they were followed by the licensing of RMP in the United States and subsequent RCTs under the auspices of the U.S. Public Health Service.

When either RMP or PZA was added to a regimen, there was a decrease in the proportion of patients with a positive 2-month sputum culture and a decrease in the relapse rate after treatment, indicating improved sterilizing activity. Furthermore, the addition of RMP to a regimen containing PZA or PZA to a regimen containing RMP also increased sterilizing activity and demonstrated the synergistic sterilizing activity of these two drugs. One regimen emerged from the numerous RCTs. It was a 6-month regimen in which RMP is given throughout, starting with 2 months of SM, INH, RMP, and PZA and is followed by 4 months of INH and RMP (2SHRZ/4HR). This regimen was pioneered in Singapore and its efficacy and low toxicity confirmed by later studies in other countries. It is now widely used with ethambutol substituted for SM (2EHRZ/4RH).

3.2. Chemotherapy of drug-resistant Tuberculosis

Most of drug-resistant Tuberculosis could be administered a standardized therapy (**Table 1**) [58,59]. However, except newly diagnosed multidrug-resistant Tuberculosis (MDR-TB), the treatment of MDR-TB is based on expert opinion and requires the creation of combination drug regimens chosen from five hierarchical groups of first-line and second-line drugs. Such therapy is associated with a high risk of intolerance and serious toxic effects. Regimens may be chosen on a standardized or empirical basis and then switched to individualized therapy after data regarding drug-susceptibility testing become available. However, reliable drug-susceptibility testing is not widely available in regions in which tuberculosis is endemic, particularly for second-line drugs. WHO treatment guidelines for multidrug-resistant tuberculosis recommend that the intensive phase of therapy be administered for at least 8 months.

A fluoroquinolone and an injectable agent should routinely be included to provide a regimen with at least four second-line drugs that will have certain or nearly certain effectiveness, as well as pyrazinamide. Such therapy should be administered for at least 20 months in patients who have not received previous treatment for multidrug-resistant tuberculosis and for up to 30 months in those who have received previous treatment.

An observational study showed that a shorter regimen, with treatment given for 9 to 12 months (the so-called Bangladesh regimen), had acceptable efficacy with fewer adverse reactions in a population with no previous exposure to second-line drugs. In 2016, WHO recommended 9-12 months MDR-TB treatment regimen under specific conditions. To be noticed, it's a conditional recommendation with very low certainty in the evidence. Now this regimen is being more widely evaluated in the ongoing Standardized Treatment Regimen of Antituberculosis Drugs for Patients with Multidrug-Resistant Tuberculosis (STREAM) trial. Since most of the recommended drugs have serious side effects that render treatment particularly difficult, expert consultation is always advised for the treatment of multidrug-resistant tuberculosis.

Extensively drug-resistant tuberculosis is extremely difficult to diagnose and treat in countries in which the disease is endemic. The condition has been associated with death rates as high as 98% among HIV-infected persons.

3.2.1. Category of Antituberculosis drug (Table 2)

3.2.1.1. Fluoroquinolones

Both levofloxacin and moxifloxacin are commonly used to treat MDR-TB. Levofloxacin is more widely available than moxifloxacin, which is more expensive although a reduction in its price is expected in the coming years.

Gatifloxacin is an affordable drug and had been commonly used by TB treatment programmes until the concerns about its dysglycaemic effects led to a global shortage in this medicine. If manufacture of quality-assured formulations of the drug restarts, it could substantially lower the costs of regimens by substituting more expensive options in fluoroquinolones.

Moxifloxacin is relatively easy to administer to older children. However, the tablet must be split to accommodate dosing in younger children and it is highly unpalatable once split or crushed. Levofloxacin is available as a suspension.

3.2.1.2. Second-line injectable agents

These agents present problems to administer intramuscularly or intravenously on a daily basis for several months, often necessitating hospitalization. Giving injections to children and underweight adults is particularly unpleasant and unwelcome.

3.2.1.3. Other agents

Ethionamide and prothionamide are inexpensive, readily available world-wide and easily administered.

Cycloserine has been one of the standard drugs for the treatment of MDR-TB for several years and therefore experience in its use is widespread. Terizidone is less widely used but is available on the GDF Products List.

Clofazimine can be difficult to procure. The implementation of these guidelines at national level needs to ensure that sufficient quantities of this medicine are available to meet the demand and that no stock-outs occur. Moreover, given that there are no good paediatric formulations the capsule contents need to be expressed manually and divided into smaller doses, with risks of incorrect dosing in children.

When linezolid is used, there needs to be close monitoring for side effects, particularly anaemia, thrombocytopenia, lactic acidosis, peripheral neuropathy and optic neuropathy, as these can be severe and life threatening. Historically linezolid has been very expensive, however, it has recently come off patient and the availability of generic products has reduced its market price substantially and it may even decrease further.

3.2.1.4. Add-on agents

Pyrazinamide is inexpensive, readily available and easy to administer.

Isoniazid is inexpensive. It is important to consider the epidemiology of high level versus low level isoniazid mutations in a population before standard treatment regimens including high-dose isoniazid are recommended.

Bedaquiline is a diarylquinoline that blocks ATPase synthesis. There has been no cross-resistance with other drugs so far. Bedaquiline has high tissue binding, which partly accounts for its half-life of more than 24 hours. The early bacterial activity is similar to isoniazid and rifampin after 5 days. For extensively drug-resistant tuberculosis, bedaquiline added to the best available baseline regimen (kanamycin,

Ofloxacin, ethionamide, pyrazinamide, and cycloserine) showed improved clearance. However, despite better bacterial clearance, the bedaquiline treatment group had more deaths than the placebo group in a comparative study. The cause of death did not fit a pattern and may be unrelated to the medication.

Delamanid, a nitro-dihydroimidazoxazole also derived from metronidazole, is effective

in replicating and nonreplicating organisms. Its MIC of 0.006–0.024 mg/ml is impressive; its intracellular activity at 0.1 mg/ml overshadows rifampin at 1–3 mg/ml. It also has delayed killing kinetics. Delamanid plus pyrazinamide and rifampin was superior to rifampin, pyrazinamide, isoniazid, and ethambutol in culture conversion in mice. In humans, delamanid is well tolerated and has good early bacterial activity.

Ethambutol is inexpensive and readily available.

PAS is available through the Global Drug Facility (GDF). Otherwise it is relatively inexpensive and easy to administer.

Amoxacillin-clavulanate is inexpensive and easily obtainable. However, the carbapenems are expensive and are difficult to administer as they must be given two or three times per day via an intravenous line.

Thioacetezone is inexpensive but it has limited availability and it is not currently available through the GDF.

3.2.2. Designing and administrating a MDR regimen

It applies to standardized and individualized regimens. The following are the basic principles involved in the treatment of MDR-TB. Early MDR-TB detection and the prompt initiation of an effective treatment are important factors in obtaining successful outcomes. The intensive phase of MDR-TB treatment should consist of at least four second-line anti-TB drugs that are likely to be effective (including an injectable anti-TB drug), as well as pyrazinamide. Where there is unclear evidence about the effectiveness of a certain drug, this drug can still be part of the regimen, however, it should not be depended upon for success. MDR regimens should include at least pyrazinamide, a fluoroquinolone, an injectable anti-TB drug, ethionamide (or prothionamide) and cycloserine. The drugs in the regimen should be judged to be "likely effective" [58]. An anti-TB drug is considered "likely to be effective" when:

– The drug has not been used in a regimen that failed to cure the individual patient;

– DST performed on the patient's strain indicates that it is susceptible to the drug (DST for isoniazid, rifampicin, Groups 2 and 3 drugs is considered reliable; DST for all other drugs is considered not reliable enough for individual patient management);

– No known resistance to drugs with high cross-resistance;

– No known close contacts with resistance to the drug;

– Drug resistance surveys demonstrate that resistance is rare to the drug in patients with similar TB history. This final criterion is relevant in the absence of DST or for drugs in which in-

dividual DST is not reliable. Note: It is not always possible that information of all five criteria can be ascertained. Therefore, clinical judgment is often necessary on whether to count a drug as "likely effective".

There are conditions when more than five drugs are used. These conditions would be applicable when the effectiveness for a drug(s) is unlikely or questionable. One such relatively common condition is the treatment of XDR-TB.

3.2.3. Treatment strategies for MDR-TB and XDR-TB

Drugs that the patient is known to have a strong contraindication of usage due to drug–drug interactions, overlying toxicities, co-morbidities, history of severe allergy or other adverse reactions, and/or pregnancy-should not be used. Every drug should be administered with a right dosage (**Table 3**). A fluoroquinolone should be used (strong recommendation, very low quality evidence). A later-generation fluoroquinolone rather than an earlier-generation fluoroquinolone should be used (conditional recommendation, very low quality evidence). In the treatment of patients with MDR-TB, ethionamide (or prothionamide) should be used (strong recommendation, very low quality evidence). This recommendation assumes the recommended drugs meet the criteria of "likely to be effective" and there are no contraindications to its use (such as severe adverse effects). The intensive phase (i.e. the initial part of treatment during which a Group 2 injectable agent is used) lasts at least eight months in total, but the duration can be modified according to the patient's response to treatment. The optimal duration of intensive phase following culture conversion, which is associated with treatment success, could not be inferred directly from the analysis used to revise the WHO programmatic management of drug-resistant TB guidelines in 2011.

Some clinical experts may prefer that the intensive phase is continued for at least four months past culture conversion. The total length of treatment is expected to be at least 20 months in most patients not previously treated for MDR-TB. Some clinical experts may prefer that total treatment be for at least 12 months past the point at which culture converts to negative and, some others may prefer not to give less than 20 months in total. Each dose is given under a patient-centered directly observed therapy (DOT) throughout the treatment. A treatment card is marked for each observed dose. DOT can be performed either at facility-based or community-based levels, keeping in mind that social support is an essential component of care and treatment delivery. Any adverse effects of drugs should be managed immediately and adequately to relief suffering, minimize the risk of treatment interruptions, and prevent morbidity and mortality due to serious adverse effects. Antiretroviral therapy (ART) is recommended for all patients with HIV and drug-resistant TB, irrespective of CD4 cell-count, as early as possible (within the first eight weeks) following initiation of the anti-TB treatment (strong recommendation). The drug dosage is usually determined by age and weight. Pyrazinamide,

ethambutol and fluoroquinolones should be given once a day. Depending on patient tolerance, once-a-day dosing is also used for oral second-line anti-TB drugs from Group 4, however, ethionamide/prothionamide and cycloserine have traditionally been given in split doses during the day to reduce adverse effects. All anti-TB drugs can be started at full dose. However, if tolerance is an issue, cycloserine, ethionamide and PAS dosing can be increased gradually over a two-week period. Injectable drugs can be given five to seven days a week depending on the availability of a skilled medical person to give the intramuscular injections. Injectable anti-TB drugs should be given once daily, i.e. do not split the dose over the day. If adverse effects are problematic in a patient, the injectable agent may be given three times a week, preferably only after culture conversion. When possible oral drugs are to be given seven days a week under directly observation. Some programmes suggest giving all drugs six days a week, but it is not known if this is equal to seven days a week. Oral drugs should not be given five days a week (only the injectable agent is allowed to be on a five days a week schedule, see above). Pyrazinamide can be used for the entire treatment. Many drug-resistant TB patients have chronically inflammed lungs, which theoretically produce the acidic environment in which pyrazinamide is more effective. Alternatively, in patients doing well, pyrazinamide can be stopped with the injectable drug if the patient can continue with at least three likely effective drugs. In MDR treatment strategies that initially enrolled patients based on their strain being resistant to rifampicin alone, isoniazid may be included in the MDR regimen until DST to isoniazid can be done to determine if the isoniazid should be continued. Patients with MDR-TB should be treated using mainly ambulatory care rather than models of care based principally on hospitalization.

3.2.4. Outcome of MDR-TB

Definitions of treatment outcomes for drug-resistant patients are as following [58]:

Cured: Treatment completed as recommended by the national policy without evidence of failure AND three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase.

Treatment completed: Treatment completed as recommended by the national policy without evidence of failure BUT no record that three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase.

Treatment failed: Treatment terminated or need for permanent regimen change of at least two anti-TB drugs because of: Lack of conversion by the end of the intensive phase; or Bacteriological reversion in the continuation phase after conversion to negative; or Evidence of additional acquired resistance to fluoroquinolones or second-line injectable drugs; or Adverse drug reactions.

Died: A patient who dies for any reason during the course of treatment.

Lost to follow-up: A patient whose treatment was interrupted for two consecutive months or more.

Not evaluated: A patient for whom no treatment outcome is assigned. (This includes cases "transferred out" to another treatment unit and whose treatment outcome is unknown).

Treatment success: The sum of Cured and Treatment completed.

For Treatment failed, lack of conversion by the end of the intensive phase implies that the patient does not convert within the maximum duration of the intensive phase applied by the programme. If no maximum duration is defined, an 8-month cut-off is proposed. For regimens without a clear distinction between intensive and continuation phases, a cut-off eight months after the start of treatment is suggested to determine when the criteria for Cured, Treatment completed and Treatment failed start to apply.

The terms "conversion" and "reversion" of culture as used here are defined as follows: Conversion (to negative): culture is considered to have converted to negative when three consecutive cultures, taken at least 30 days apart, are found to be negative. In such a case, the specimen collection date of the first negative culture is used as the date of conversion. Reversion (to positive): culture is considered to have reverted to positive when, after an initial conversion, two consecutive cultures, taken at least 30 days apart, are found to be positive. For the purpose of defining Treatment failure, reversion is considered only when it occurs in the continuation phase.

Table 1: Standardized regiment for drug-susceptible and drug-resistant tuberculosis

Category	Recommended Regimen	Lowest duration
Drug-susceptible[a]	2HRZE/4HR	6 months
Resistant to H(±S)	RZE±FQ[b]	6-9 months
Resistant to H+E(±S)	RZFQ	9-12 months
Resistant to H+E+Z(±S)	RFQPto+SLIA 2-3 months	18 months
Resistant to R	MDR-TB regimen +H	20 months
MDR-TB[c]	8ZAk(Cm)Mfx(Lfx)Pto(CS)Cfz(CS)/12 ZMfx(Lfx)Pto(CS)Cfz (CS)	20 months

Abbreviations:

[a] For extrapulmonary tuberculosis, treatment should last at least 1 year.
[b]Fluoroquinolone
[c]Newly-diagnosed MDR-TB

Table 2

Drug	Weight(kg)			Maximam dosage	Frequency
	<33a	<50	≥50		
Isoniazid (H)	10mg/kg	300mg	300mg	300 mg	qd
Rifampin (R)	10-20mg/kg	450mg	600mg	600mg	qd
Ethambutol (E)	15-25mg/kg	7500mg	1000mg	1500mg	qd
Pyrazinamide (Z)	30-40mg/kg	1500mg	1750mg	2000mg	qd or tid
Rifabutin (Rfb)	-b	150-300 mg	300mg	300mg	qd
Rifapentine(Rpt)	10-20mg/kg	450mg	600mg	600mg	biw
Streptomycin (S)	15-20mg/kg	750mg	750mg	1000mg	qd
Amikacin (Ak)	15mg/kg	400mg	600mg	800mg	qd
Capremycin (Cm)	15-20mg/kg	750mg	750mg	1000mg	qd
Levofloxacin (Lfx)	10mg/kg	400mg	500-600mg	750mg	qd
Moxifloxacin (Mfx)	7.5-10mg/kg	400mg	400mg	400mg	qd
Gatifloxacin (Gfx)	-	400mg	400mg	400mg	qd
Prothionamide (Pto)	15--20mg/kg	600mg	800mg	800mg	bid or tid
Cycloserine (Cs)	15-20mg/kg	500mg	750mg	1000mg	bid or tid
p-aminosalicylic acid (P)	200-300mg/kg	8g	10g	12g	qd
Linezolid (Lzd)	-	600mg	600mg	1200mg	qd
Clofazimine (Cfz)	-	100mg	150mg	300mg	qd
Amocixilline/Clavulate (Amx/Clv)	50 mg/kg	1300mg	1950mg	-	bid or tid
Clarithromycin (Clr)	7.5mg/kg	500	750	1000	bid or tid
Imipenem (Imp)	60mg/kg	1500	2000	2000	bid or tid

Abbreviations:

[a]for children and adults whose weight are less than 33 kg.

[b]no data

Dosage and frequency of antituberculosis drug. All patients should be administered with drugs based on their weight.

Table 3: Medicines recommended for the treatment of rifampicin-resistant and multidrug-resistant TB[1]

A. Fluoroquinolones[2]	Levofloxacin	Lfx
	Moxifloxacin	Mfx
	Gatifloxacin	Gfx
B. Second-line injectable agents	Amikacin	Am
	Capreomycin	Cm
	Kanamycin	Km
	(Streptomycin)[3]	(S)

C. Other core second-line agents[2]	Ethionamide / Prothionamide		Eto / Pto
	Cycloserine / Terizidone		Cs / Trd
	Linezolid		Lzd
	Clofazimine		Cfz
D. Add-on agents (not part of the core MDR-TB regimen)	D1	Pyrazinamide	Z
		Ethambutol	E
		High-dose isoniazid	Hh
	D2	Bedaquiline	Bdq
		Delamanid	Dlm
	D3	p-aminosalicylic acid	PAS
		Imipenem-cilastatin[4]	Ipm
		Meropenem[4]	Mpm
		Amoxicillin-clavulanate[4]	Amx-Clv
		(Thioacetazone)[5]	(T)

1. This regrouping is intended to guide the design of conventional regimens; for shorter regimens lasting 9-12 months the composition is usually standardized

2. Medicines in Groups A and C are shown by decreasing order of usual preference for use

3. Resistance to streptomycin alone does not qualify for the definition of extensively drug-resistant TB (XDR-TB)

4. Carbapenems and clavulanate are meant to be used together; clavulanate is only available in formulations combined with amoxicillin

5. HIV-status must be tested and confirmed to be negative before thioacetazone is started

3.3. Treatment of extrapulmonary Tuberculosis

In most cases of extrapulmonary tuberculosis there are many fewer organisms present. In general, regimens used for pulmonary tuberculosis are effective in the treatment of extrapulmonary tuberculosis. WHO recommends classification of the disease into severe and non-severe forms. Severe forms include meningeal and central-nervous-system tuberculosis, spinal tuberculosis, abdominal tuberculosis, bilateral pleural effusion, pericardial effusion, and bone and joint tuberculosis involving more than one site. All major organisations agree that some forms of disease, such as meningitis, may benefit from a longer treatment course. Steroids

should be used for patients with meningitis, particularly with neurological impairment, since these drugs are likely to decrease morbidity and mortality in such cases. A RCT performed in Vietman show that adjunctive treatment with dexamethasone improves survival in patients over 14 years of age with tuberculous meningitis but probably does not prevent severe disability [60]. However, another RCT show in patients with tuberculous pericarditis, neither prednisolone nor M. indicus pranii had a significant effect on the composite of death, cardiac tamponade requiring pericardiocentesis, or constrictive pericarditis [61].

3.4. Surgery for Tuberculosis

Surgery has been employed in treating TB patients since before the advent of chemotherapy. In many countries it remains one of the treatment options for TB. With the challenging prospect in many settings of inadequate regimens to treat multidrug and extensively drug-resistant TB, and the risk for serious sequelae, the role of pulmonary surgery is being re-evaluated as a means to reduce the amount of lung tissue with intractable pathology, to reduce bacterial load and thus improve prognosis.

Indications for surgery include: persistently positive smear or sputum culture for acid-fast bacilli despite aggressive chemotherapy; high risk of relapse (based on drug resistance profile and radiological findings); complications of tuberculosis including bronchiectasis, empyema, haemoptysis; sufficient drug treatment available (to reduce bacterial burden and allow healing of bronchial stump) [58].

Preoperative work-up include: chest CT scan to assess extent of disease and guide surgical resection; pulmonary function testing; and ventilation perfusion scan to ensure presence of adequate pulmonary reserve to tolerate surgery; bronchoscopy to rule out endobronchial tuberculosis, contralateral disease, and malignancy; echocardiogram to rule out heart failure and pulmonary hypertension; nutritional assessment to ensure patient can tolerate and recover from surgery.

4. Future Perspectives

Although a great deal of progress have been achieved in the past decades. However, no new first-line drugs have been discovered for several decades. The influence of HIV infection on the tuberculosis burden and MDR-TB will be difficult to reverse. Further progress will require continued rigorous and dedicated application of current technology and will be greatly facilitated by the discovery and widespread application of new diagnostic techniques, drugs, and prevention strategies, such as an effective vaccine. There is still a long road to eliminate TB. The future diagnosis should be some high accuracy methods with less turnout time, and the future treatment should be short and less interactive with other drugs (such as anti-retrovirus agent) and effective vaccine.

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