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Review Article

Clinical Biology for Effective Treatment

An Overview of Extra Pulmonary Tuberculosis in Smear Negative Cases and their Analysis

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Abstract: *Mycobacterium tuberculosis* (MTB), a bacterium which causes the tuberculosis disease, is one of the most trounce disease in the country. It mainly affects the lungs and other parts of the body. Currently smear-positive cases are further confirmed through chest radiography for PTB. These cases are further subjected for short course chemotherapy (SCC), followed by first line primary drugs, a scheme undertaken by National Tuberculosis Programme to overcome PTB infection. However, the identification of infection through smear-negative results are challenging as the cases may become positive over the time and progresses to cause the Extra Pulmonary Tuberculosis (EPTB). Such infections are asymptomatic and referred to as latent infection, where, MTBremains stable for many years at a specific site and later activates. Worldwide about 10-25% of latent infections progress to an active disease and if undiagnosed, the mortality rate may reach up to 50%. Twenty five percent of EPTB infection differs from organ to organ of the body. However, in such cases, diagnosis may be elusive and is usually delayed. The proper identification of infection that strongly focuses on the infection of either i.e., TB or EPTB is the need of the hour. The diagnosis of EPTB will be elusive, necessitating a high index of suspicion over TB or EPTB. However, smear-negative cases may be positive for EPTB infection, where the suspects are to be further subjected for molecular methods of EPTB detection and confirmation.

Keywords: Tuberculosis, Extra Pulmonary tuberculosis, Miliary, Genitourinary, Meningitis, Peritonitis Pericarditis, Lymphadenitis, Cutaneous, Liver, and Smear-Negative Case Analysis etc.,

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1. INTRODUCTION

Tuberculosis has been a global health concern from the ancient time especially in a developing country like India. India has recorded highest reported positive cases in tuberculosis incidence according to the World Health Organization (WHO) and over 10.2 million new cases diagnosed every year globally in 2019[1]. India has recorded the highest TB cases of 2.69 million of which 14% are EPTB, 10% are multi drug resistant (MDR) and 10% (XDR-TB) in 2019.TB is caused by the *Mycobacterium tuberculosis*, that affects the lungs commonly called as Pulmonary TB (PTB) and it also affects the other organs of the body viz., central nervous system, abdomen, skin, kidney etc., called as Extra Pulmonary TB (EPTB) [2, 3]. The *MTB* enters alveoli through the respiratory system and infects the lungs. Later it remains dormant for many long years and becomes active when the immunity is lowered; it slowly re-activates and affects lymph nodes of the body. From the affected lymph nodes through the bloodstream it spreads to different organs of the body (listed below Table 1). As per the reports of Revised National Tuberculosis Centre Programme (RNTCP) tuberculosis is considered as re-emerging disease globally. Pulmonary TB is identified by the acid-fast staining of sputum samples; but EPTB is noticed in different organs of the body at a very later stage of latent infection by MTB[7-8]. EPTB has remained the major public health problem nowadays.Despite the availability of modern techniques of diagnosis and effective drugs, India is still the highest with one-fourth of the world's EPTB disease burden. New interventions are needed to interrupt the transmission and spread of the EPTB and eradicate the disease. It is estimated that every two minutes a person dies from TB in India. Keeping this in mind, the initiative was taken to carry out an extensive review of the current literature on the subject of pathogenesis. A large number of clinical specialists from different fields and experts from India and abroad have worked tirelessly for the past one year to bring out evidence-based, comprehensive guidelines on the management of all forms of MTB in EPTB [9,10].

Table 1: Different types of Extra Pulmonary Tuberculosis (EPTB) include

Type of the EPTB	Pathogenesis & Severity	Organs affected	Clinical Symptoms
TB Lymphadenitis	Tuberculous lymphadenitis (scrofula) typically involves the lymph nodes in the posterior cervical and supraclavicular chains[7]. In a pulmonary disease where the Mediastinal lymph nodes are commonly enlarged at primary stage. Infections in this stage are common due to continuous spread of the intrathoraciclymphatics and also infection in the tonsils and adenoids[7].	Lymph Nodes	Cervical tuberculous lymphadenitis is characterized by progressive swelling of the affected nodes[7,8].
Miliary TB	Also known as generalized hematogenous TB. It occurs when a tuberculous lesion erodes into a blood vessel, disseminating millions of tubercle bacilli into the bloodstream and throughout the body. It is commonly seen in children below four years with immune-compromised state [7,8].	Blood Stream	Symptoms are fever, chills, weakness, malaise and dyspnoea. Bone marrow involvement may cause anemia, thrombocytopenia, or a leukemoid reaction[7,8].
Genitourinary TB	Infection of the kidneys may manifest as pyelonephritis (eg, fever, back pain, pyuria) without the usual urinary pathogens on routine culture (sterile pyuria) In these cases, infection may spread to the perinephric space and down the psoas muscle, sometimes causing an abscess on the anterior thigh. Salpingo-oophoritis can occur after menarche, when the fallopian tubes become vascular[7,8].	Genitourinary	Symptoms include chronic pelvic pain and sterility or ectopic pregnancy due to tubal scarring[7,9].
TB Meningitis	The most common in the elderly and immune-compromised people, but in regions where TB is common among children below 5 years[7]. It is the infection of tissues covering the brain and spinal cord (Meninges) [7].	Brain and Spinal Cord	Symptoms are low-grade fever, unremitting headache, nausea, and drowsiness, which may progress to stupor and coma. It is one form of the TB that can be prevented in childhood by vaccination with BCG[11, 12].
TB Peritonitis	Peritoneal infection represents seeding from abdominal lymph nodes or from salpingo-oophoritis[13, 14]. Peritonitis is particularly common among alcoholics with cirrhosis	Adominal region	Symptoms may be mild, with fatigue, abdominal pain, and tenderness, or severe enough to mimic acute abdomen[7,11]
TB Pericarditis	Pericardial infection may develop from foci in mediastinal lymph nodes or from pleural TB. In some high-incidence parts of the world, TB pericarditis is a common cause of heart failure [15]	Mediastinal Lymph Nodes	The patients also have pericardial friction rub, pleuritic and positional chest pain and fever too[16].
TB of Bones and	Joints are weight bearing are most knowingly	Bones and Joints	Symptoms include progressive

Joints	involved, and also bones of wrist, elbow hand feet may also be affected, especially, after an injury or surgery[17]. Spinal infection causes the Pott's disease that starts with a vertebral body and adjacent vertebrae.		or constant pain in involved bones and chronic or subacute arthritis (usually monoarticular) [18, 19].
Cutaneous Tuberculosis	It is also known as scrofuloderma that results from the direct examination of the TB, mainly focus on lymph nodes, an infected bone or joint that leads to ulcer and sinus tracts. The sensitized patients are developed with the Lupus vulgaris from hematogenous to the skin of an extracutaneous[20]. It rarely develops on the abraded skin in patients with cavitary pulmonary TB[11-12].	Skin	Symptoms include night sweats, persistent coughs.[11] Loss of appetite, unusual weight loss, fever, general fatigue[12]
Gastrointestinal (GI) TB	Because the entire GI mucosa resists TB invasion, infection requires prolonged exposure and enormous inocula. Ulcers of the mouth and oropharynx may develop from eating *M. Bovis* - contaminated dairy products. Intestinal invasion generally causes hyperplasia and inflammatory bowel syndrome [8, 9]. By this it can turn to ulceration and fistulas. Hyperplasia is generally caused by an intestinal invasion with an inflammatory bowel syndrome that provokes severe pain, diarrhea, obstruction and hematochezia[10, 11].	Intestine	Symptoms of obstruction, right iliac fossa pain, or a palpable mass in the right iliac fossa, abdominal pain, fatigue, [7,8,11]
TB of the Liver	Liver infection is common in patients with advanced pulmonary TB and widely disseminated or Miliary TB[14]. It occasionally spreads to the gallbladder, leading to obstructive jaundice. However, the liver generally heals without sequelae when the principal infection is treated [20]. TB in the liver occasionally spreads to the gallbladder, leading to obstructive jaundice. Necrotizing granuloma is most frequently obtained from liver samples (90-100%) rather than bone marrow (31-82%) or transbronchial biopsy (63-72%)	Liver	The most common presentation of hepatic TB is abdominal pain, hepatomegaly, jaundice, fever, and chills. Alkaline phosphatase elevations and hyponatremia usually are prominent features. In 65% to 78% of patients with hepatic TB, respiratory symptoms, or chest radiographs suggest pulmonary TB[14, 20].
Laryngeal Tuberculosis	Laryngeal tuberculosis usually entails the development of masses, ulcers or nodules in the larynx and vocal cords, which are usually mistaken as laryngeal neoplasms. The most common clinical manifestation is dysphonia but it can also produce coughing, stridor, and hemoptysis. It is usually associated with concomitant pulmonary TB, and it is thus a highly bacciferous and contagious form of the disease [21, 22].	Mouth	Symptoms of laryngeal tuberculosis are dysphonia, weight loss, cough, dysphagia, and odynophagia. Hoarseness and fatigue may be present as well[21].
Central Nervous System (CNS)	When TB occurs in the tissue surrounding the brain or spinal cord, it is called tuberculous meningitis. Tuberculous meningitis is often seen at the base of the brain on imaging studies. Symptoms include headache, decreased level of consciousness, and neck stiffness[23]. The duration of illness before the diagnosis is variable and relates in part to the presence or absence of other sites of involvement. [24, 25].	Nervous System	Symptoms are Severe headache, Back pain, Stiff neck, Confusion. Weakness, Fever, Seizures, Paralysis[23, 24].
Other sites	Rarely, TB develops on abraded skin in patients with cavitary pulmonary TB[4, 5]. TB may infect the wall of a blood vessel and has even ruptured the aorta. Adrenal involvement, leading to Addison disease, formerly was common but now is rare. Tubercle bacilli may spread to tendon sheaths (tuberculous tenosynovitis) by direct extension from adjacent lesions in bone or hematogenously from an infected organ [6, 18].	liver, spleen, intestines, kidney, abdomen, and bladder	Common symptoms are coughing that lasts three or more weeks, coughing up blood, chest pain, or pain with breathing or coughing, unintentional weight loss, Fatigue, fever, night sweats, chills[6,5,18].

The TB is re-emerging in the present days and along with this there is also an increase in the EPTB, which is because of the neglected sputum–negative cases, where the MTB will be dormant for the several years, and as the patient's immune system fades, it reactivates moves to the other organs of the body & hence the neglected sputum-negative cases are becoming more and more challenge in the current situation and needs to be further directed towards molecular diagnosis followed by confirmation and treatment.

1.1 PATHOGENESIS OF EXTRA PULMONARY TUBERCULOSIS

TB infection occurs when a person inhales droplet nuclei containing tubercle bacilli that reach the alveoli of the lungs. These tubercle bacilli are ingested by alveolar macrophages; the majority of these bacilli are destroyed or inhibited. A small number may multiply intracellularly and are released when the macrophages die [26, 27]. If viable, these bacilli may spread by way of lymphatic channels or through the bloodstream to more distant tissues and organs (including areas of the body in which TB disease is most likely to develop: regional lymph nodes, the apex of the lung, kidneys, brain, and bone). This process of dissemination primes the immune system for a systemic response towards the tuberculosis bacterium.Further details about the pathogenesis of latent tuberculosis infection (LTBI) and TB re-infection disease involves the study, where, the patients will have bacterium present in their body in a dormant stage where LTBI is established once the immune system is low it reactivates. Tuberculin skin test (TST) and Interferon-gamma assay (IGRA)determines LTBI. It can take 2 to 8 weeks after the initial TB infection for the body's immune system to react to tuberculin and for the infection to be detected by the TST test and IGRA test. Once a person is been diagnosed with LTBI, the person will remain alert for symptoms of the active tuberculosis. Further the person shall be under the treatment of first line anti-TB drug.After completing the full course of medication, there is no guarantee that the tuberculosis bacteria has all been killed, the bacterium may remain latent, later reactivates and then, the person may develop active TB. Within weeks after reactivated infection, the immune system is usually able to halt the multiplication of the tubercle bacilli, preventing further progression[28, 29]. However, the bacteria spread outside the lungs and infects the lymph node. From lymph node the M. tuberculosis is transferred through the blood to the other organs of the body.Fig 1Shows the transformation of M. tuberculosis to Extra Pulmonary Tuberculosis process.

1.2 *RISK OF LATENT TB INFECTION (LTBI) PROGRESSING TO TB DISEASE*

The person who has LTBI can develop TB disease later, and some people may have a higher TB risk. The HIV, Diabetics etc., patients have the highest risk of developing TB disease due to weakened immune system and presence of the LTBI. There are about 7-10% of the patients with HIV co-infection. In accordance to this, children <5 years of age have a chance of LTBI or TB disease [30]. Drug-resistant TB is transmitted in the same way as the drug-susceptible TB [31-32]. However, delay in the recognition of drug resistance or prolonged periods of infectiousness may facilitate increased transmission and further development of bacteria for TB infection. Drug resistance exhibited by the bacteria may be due to its exposure to first line anti-TB drugs viz., rifampicin. To suppress the bacterium, combinations of other drugs along with or without rifampicin are prescribed. Interactions between rifampicin and other drugs in combination results with induced pathways that metabolize other drugs reducing its concentration and its effects, where the rifampicin dominates. To balance the therapeutic effect, the dosage of riafmpicin and other drugs in combination needs to be the same. However, once the rifampicin is discontinued, its metabolism inducing effect resolves within about 2 weeks and followed by the dosages of other drugs needs to also be reduced (American Thoracic Society; CDC 2003).In countries with comprehensive diagnostic and reporting systems, Globally, notified EPTB cases (without concurrent pulmonary involvement) comprises of 14% (new and relapse) in 2017 (NICE 2016) with specific forms of EPTB viz., lymphatic, pleural, and bone or joint disease, which is the most common, while pericardial, meningeal and disseminated (miliary) forms are more likely to result in a fatal outcome. Many TB patients have concomitant illnesses [34, 35]. A treatment for EPTB includes; antibiotics, for pericarditis and meningitis, sometimes corticosteroids & sometimes surgery. Drug treatments are the most important modality and follows standard regimens and principles of TB First-line drugs like isoniazid, rifampicin, ethambutol that are included for six to nine months of therapy, which is probably an adequate for most infected sites except the meninges, which require treatment for 9 to 12 months. Corticosteroids may help in pericarditis and meningitis. Bacterium may exhibit drug resistance to first line anti TB drugs that are prescribed as preliminary treatment. Drug resistance is a major concern which is increased by poor adherence and inadequate susceptibility testing [25, 26]. Surgery is suggested further, as a result of failure in anti TB drug activity due to increased resistance, to drain empyema, cardiac tamponade or central nervous system (CNS) abscess, to close bronchopleural fistulas, to resect infected bowel and to decompress spinal cord encroachment. Surgical debridement is sometimes needed in Pott's disease is a combination of osteomyelitis and arthritis that involves the vertebra to correct spinal deformities or to relieve cord compression if there are neurologic deficits or if pain persists. Fixation of the vertebral column by the bone graft is required in only the most advanced cases. Surgery is usually not necessary for TB lymphadenitis except for diagnostic purposes. [36, 37]

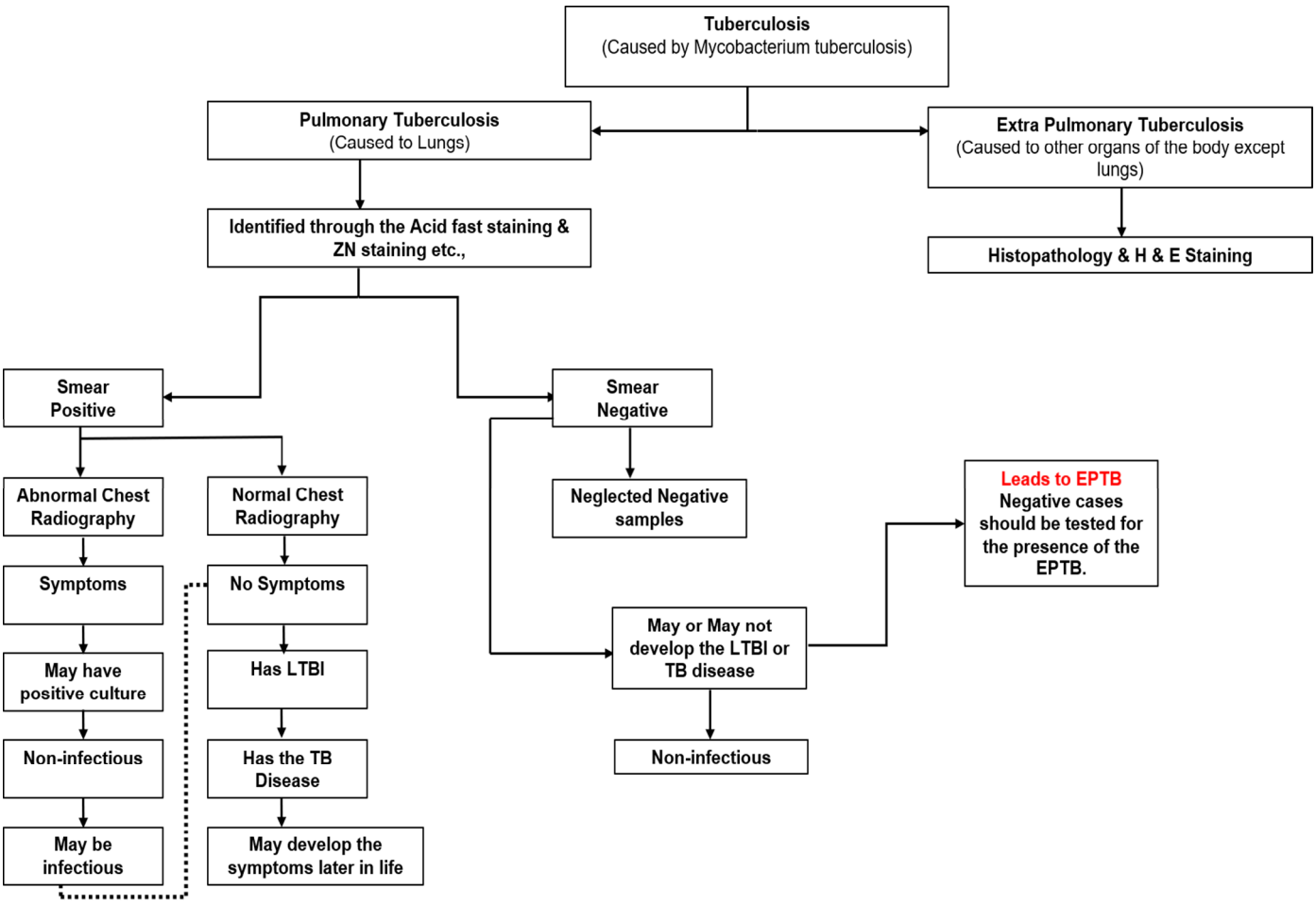

Fig 1: Flowchart shows the transfer of MTB and leading to EPTB

1.3 DETECTION AND DIAGNOSIS

Diagnosis is similar to that for pulmonary TB. The TB diagnosis includes Radiography, AFB smear microscopy and Culture, Line probe assay, Nucleic acid amplification testing, Xpert MTB/RIF (Cepheid), Chest X-ray, TST or IGRA, Microscopic analysis(with appropriate staining) **etc.,** and mycobacterium cultures of affected body fluids (CSF, urine, or pleural, pericardial, or joint fluid) and molecular method of tissue for mycobacteria[31]. Nucleic acid–based testing can be done for both on fresh fluid (fresh biopsy samples) and fixed tissue (example: if MTB was not suspected during a surgical procedure and cultures were not included). Blood culture results are positive in about 50% of patients with disseminated EPTB; such patients are often immune-compromised, often infected with HIV, diabetics etc.,. However, cultures and smears of body fluids and tissues are often negative because few organisms are present; in such cases, nucleic acid amplification tests (NAAT) may be helpful for the MTB detection [15].

- Typically, lymphocytosis is present in body fluids. A very suggestive finding in the CSF is a glucose level < 50% of that in serum and an elevated protein level.
- If all tests are for EPTB are negative and miliary TB is still a concern, biopsies of the bone marrow and the liver are subjected for histopathological studies. If MTB is highly suspected based on other features (Eg; the granuloma on biopsy, positive TST or IGRA plus unexplained lymphocytosis in pleural fluid or CSF), treatment should usually proceed despite an inability to demonstrate TB bacterium.
- Chest x-ray and other imaging, TST, and IGRA can also provide helpful diagnostic information.
- The chest x-ray may show signs of primary or active TB; in miliary TB, it shows thousands of 2 to 3mm interstitial nodules evenly distributed through both lungs.
- Other imaging tests are done based on clinical findings. Abdominal or GU involvement usually requires CT or ultrasonography; renal lesions are often visible. Bone and joint involvement require CT or MRI; MRI is preferable for spinal cord disease [32].
- TST and IGRA may initially be negative, but a repeat test in a few weeks is likely to be positive. If it is not, the diagnosis of TB should be questioned or found causes of energy sought [33].

The bacterium exhibiting resistance to any two drugs is referred as drug-resistant and for more than two drugs is referred as multi-drug resistant mycobacterium tuberculosis (MDR-MTB). The two most important first-line drugs for TB treatment are isoniazid & rifampicin, if bacterium is resistant to these two drugs then it leads to the MDR-TB. Similarly XDR-TB defines as the invitro resistance towards isoniazid & rifampicin including fluoroquinolone and to least anti-TB drugs among the second-line injectable drugs like capreomycin or kanamycin & amikacin[33-35].The analyses of the drug resistance are shown in the Figure 2. From the in vitro studies of Giovanni Sotgiuet. al., (2010) MDR & XDR-TB resistance exhibited by the bacterium was found suppressed with the addition of linezolid to the first and second line combination of anti-TB drugs. Their main work included the linezolid for the treatment of the MDR and XDR TB. The

study revealed 33% positive result [83], parallelly 50% in a study conducted in Europe [82], 33.3% in Asia [83] & 16% in USA[84] and many of the studies were performed individually with the patients at University level along with the patients at tertiary hospitals. Around 10 out of 12 clinical samples were evaluated in the current analysis for the use of linezolid at 600mg/day concentration majorly for treating the MDR & XDR TB. Alffenaa et. al., (2010) study demonstrated with the concentration of the linezolid of about 300mg/day concentration with the minimum inhibitory concentration (MIC) i. e. 0.125-0.5mg/l against M. tuberculosis [85], this was done because in their study 600mg/day concentration was recording over dosage and to prevent peaks of the blood which was responsible for non-hematological adverse events. These resulted with drug toxicity and the dosage was discontinued for the MDR-TB. Without analyzing the stage of the infection the drugs are prescribed, which is also causing the toxicity of about 0 to 8.2%, as per the analysis of the DTOS-Plus pilot projects. MDR & XDR-TB level is very high and the government is taking all types of precautions to eradicate TB in the future, but M. tuberculosis is increasing more and more currently. There are also many recent techniques and methods in TB diagnosis, one of them includes cartridge-based nucleic acid amplification test (CBNAAT) with a high potential to diagnose TB and rifampicin resistance within 2 hours. The alternate method is tuberculo stearic acid test (TBSA) from the clinical samples to detect cell wall fatty acid of Mycobacterium. The TBSA detection of samples of CSF is the best and rapid method for the diagnosis of the MTB and other MTB Complexes, like TB meningitis, peritoneum etc., [86] The Latex particle agglutination assays and reverse passive haemagglutination assay (RPHA) have been used mainly for agglutination reactions. Which is mainly sensitized with a monoclonal antibody directed against lipoarabinomannan (LAM) used to detect the TB and TB meningitis[87], It also goes with Enzyme Linked Immunosorbent Assay (ELISA) based methods, Multiplex PCR, Normal PCR; the molecular methods; probe based techniques, Random amplification of polymorphic DNA (RAPD) methods, transcript mediated assay etc.,

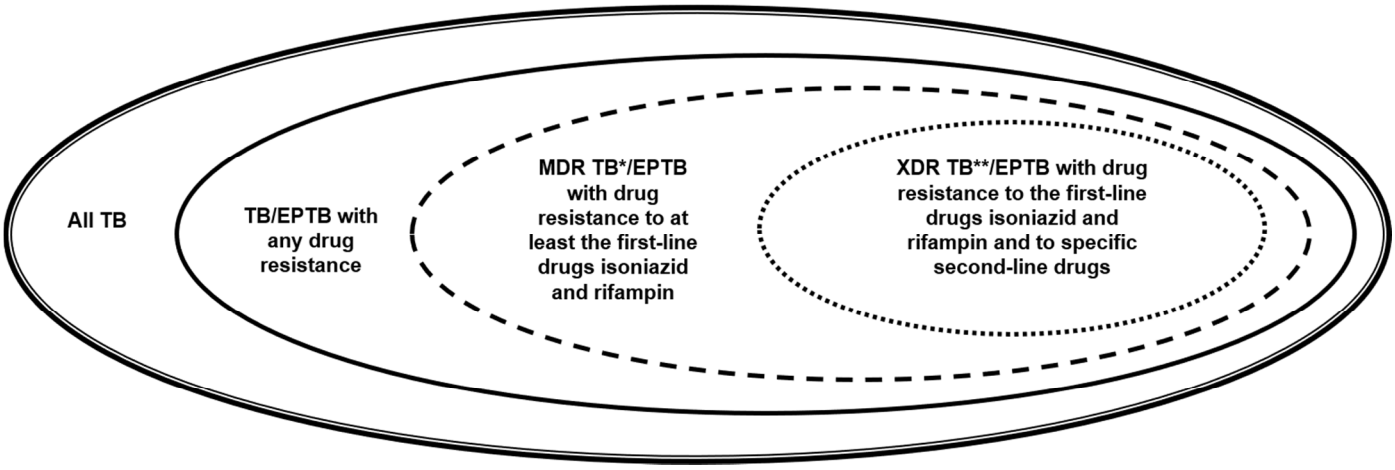

Fig 2: The following figure explains the drugs which are resistances to MTB at different levels and sites of infection.

1.4 CYTOKINE BIOMARKERS FOR THE DIAGNOSIS OF THE TUBERCULOSIS INFECTION

The diagnosis of TB has limitations in skin tuberculin test and interferon gamma assay. This study goes with the investigation of the *M. tuberculosis* specifically through the cytokine biomarkers for the MTB detection in children and adults. Advanced studies for the TB detection among the children and adults are specifically investigated through the use of cytokine biomarkers. In order to get accurate diagnosis of MTB in vitro immunodiagnostic tests, and interferon-gamma release assays (IGRAs), can be performed, that shows high specificity [88]. They also have the disadvantages, of low sensitivity for active MTB detection, especially in the immunocompromised patients and children below 5 years. Antigen stimulation of the cytokine response will be the suitable technique for the mycobacterium detection through the immunodiagnostic test that mainly differentiate between the tuberculosis-infected and tuberculosis-uninfected individuals, and also helps in the identification between LTBI and Active tuberculosis. Many tests and assays have showed negative results in the MTB detection to identify TB at a specific level, where the cytokines methods are found effective in detection. Antigens are generally low in the MTB infected patients and there is a necessity for the serological tests for TB diagnosis, prognosis, and treatment monitoring. Some of the specific serological tests involves CD4+ T cells that correlate with the MTB antigen that acts as biomarkers for predicting TB disease outcomes and also with disease mechanism[89].Current review helps in understanding the recent advances on TB biomarkers, particularly host biomarkers that mainly have the potential to diagnose active TB and LTBI, where these two types of TB are prerequisites for many active TB and EPTB cases in the Country. Hence the main care should be taken for the treatment of the active TB and LTBI at the preliminary stage. This Cytokines method gives about 75% of the accurate results and can be implemented to the TB diagnosis[90]. National and International tuberculosis (TB) control programs are established for the detection and prevention of smear-positive pulmonary TB. But the Smear-Negative Pulmonary TB (SNPT) is common currently [36]. In some particular developing countries like India are affected by dual infection viz., active TB and TB/negative pulmonary TB [38, 39].

1.5 THE SPUTUM NEGATIVE CASE STUDY

The positive sputum cases are confirmed as pulmonary tuberculosis. The negative cases of sputum are neglected in the current scenario so that it is leading to the LTBI and which is finally causing EPTB[39]. The smear-negative pulmonary

TB (SNPT) is increasing its cases along with co-morbidity and mortality. Studies on smear-negative PTB cases for 2 years (2017-19) revealed that the presence of 76% of PTB, 35% of LTBI, where EPTB accounted for 24% in which 14-15% were SNTB [40, 41].

1.6 SPUTUM INDUCTION PROCEDURE

The test negative-sputum induction will be performed by respiratory clinic & research centers with Biosafety Lab 3 (BSL3) enclosed with pressure induction booth [42].

1.7 LABORATORY METHODS FOR EXAMINING THE SPUTUM SAMPLES

The sputum samples were subjected for the Acid fast stain (AFB), auramine O-stain, ZN staining etc., Later, the sputum samples were decontaminated with N-acetyl-L-cysteine/ sodium hydroxide and then centrifuged for the pellets, at the same time 0.5 mL of the deposit was inoculated into a Mycobacterium Growth Indicator tube 960 (protocol as per Becton Dickinson Diagnostics, Franklin Lakes, NJ, USA) [47] for culturing the bacterium. AFB staining is performed before decontamination process so that it reveals the bacterial load [48, 49]. Acid-fast bacilli give the culture-positive results that are identified as M. tuberculosis complex. Apart from culturing the bacterium, the decontaminated samples can be subjected for molecular analysis directly (PCR based, MTB detection or Genotype MTB DR pluss assay) [50] (version 1; Hain Life Sciences, Nehren, Germany). The MTBDR plus assay testing is suggested and performed for routine drug susceptibility testing at a later stage of infection [51, 52].

1.8 OCCURRENCE AND HISTORY OF EPTB WITH HIV PATIENTS

The main understating of this study is smear-negative pulmonary TB is based on the determination of the pre-HIV and HIV positive patients with co-morbidity. Murray et al., (1990) reported that the data collected from the United States estimated 1.22% cases of smear-negative EPTB; each smear-negative case developed TB in some major high burden countries [51]. The smear-negative cases have joint co-morbidity with the HIV positive ones, this is because when the person infected with the TB the immune response is reduced in the body and the patient infected with HIV have the chance of TB infection. Both HIV and smear-negative TB is more commonly found in adults and children below <5 years. One of the studies revealed that0-14 year's age group showed about 10% cases from smear-negative TB which was mainly found in US (Bell DJ et. al., 2009) [91]. Similar studies from the Norway revealed 6% of positive cases in smear tests [54]. The overview or a graphical representation of the smear-negative TB from the age group of 0-6, above 6 and adults are shown in the Figure 3. The children with primary infection show the low smear-positive results for TB that was considered as smear-negative [52, 53]. Pediatric illness can also cause major health problem diseases, collecting samples from children with such disturbance leads to misdiagnosis. For the detection of the smear staining is around 5000 to 10,000 acid-fast bacillus (AFB) per milliliter of sputum is required. But for culturing requires only 10 to 100 viable bacterial cells [55, 56]. Due to this the results revealed smear-negative & culture-positive & the patient have minimal disease with a low bacillary count. InUS study, smear-negative cases have shown positive for the bacterium from the maximum bacterial count [36]. Similarly, from smear-negative cases, 60-80% were with minimal bacterial count, 30-40% were with maximum bacterial count and 5-10% with heavy bacterial count (cavitary lesions). The smear-negative association study should be minimal in the pulmonary for the lesions that would imply the infective and mortality of the disease rate. Based on the bacterial count, the disease can be diagnosed as infective and the disease rate can be analyzed [37, 38]. The smear-positive cases can be varied according to the prevalence of the study in children < 5years that varied between 39-65%, whereas tuberculin rate was only 4.7-26.8% among the smear-negative patients [39, 40]. Mainly the Radiography diagnosis is used for the positive of TB. The Narain R et. al., (1968), DNA fingerprinting study revealed the attribute about 17% of low transmission of the smear-negative and culture-positive pulmonary TB [41, 42]. The mortality level of HIV negative & smear-negative is very low. The Longitudinal survey was conducted in Bangalore city, Karnataka in between 1961 and 1963, where, untreated TB patients were found with a high mortality rate and follow-up was for 18 months with 34.7% smear-positive,[43, 44] 14.1% smear-negative and 2-3% culture-positive cases are noticed that drastically increased the disease incidence in City, and the radiological diagnosis was 5% for smear-negative culture-negative cases [45, 46].In the Hong Kong research study (Chest Research Centre, 1981) on smear-negative cases revealed that a total of 283 cases of smear-negative patients were assigned for about 30 months with chemotherapy randomly. In this, only active cases of culture study were confirmed and the remaining diagnoses were by radiography, clinical deterioration etc.,[41, 42]. A total of 71% of smear-negative patients had developed active TB. Noticing that nearly half of the smear-negative cases required the treatment for active TB within 3 months. Some smear-negative cases should be followed-up for for the early detection of TB or EPTB. When comparing the survey of the Bangalore city, Karnataka (Narain R et. al., 1968), with Hong Kong study (Chest Research Centre, 1981) overall 50% of the 457 patients are the radiographic suspension for TB, but smear-negative cases were processed to the active TB disease within 12 months[39]. Finally, smear-negative pulmonary TB and HIV can be successfully treated with a wide variety of regimens [40, 41]. For example, a study in Hong Kong found(Chest Research Centre, 1981) that treatment with streptomycin (S), ionized (H), rifampicin (R), and pyrazinamide (Z) for 4 months cured all 293 patients with smear-negative pulmonary TB and had a relapse rate of only 2% after 5 years [45]. Longer, cheaper, less-intensive therapies were found effective. The World Health Organization (WHO) therefore recommends one regimen for Category I smear-positive patients and a different treatment for Category III smear-negative cases who are not severely ill and do not have extensive parenchymal involvement but has the MTB infection. African studies from the pre-HIV era have justified the treatment of smear-negative cases with cheaper, less aggressive 12-month regimens of secondary line drugs [48, 49].

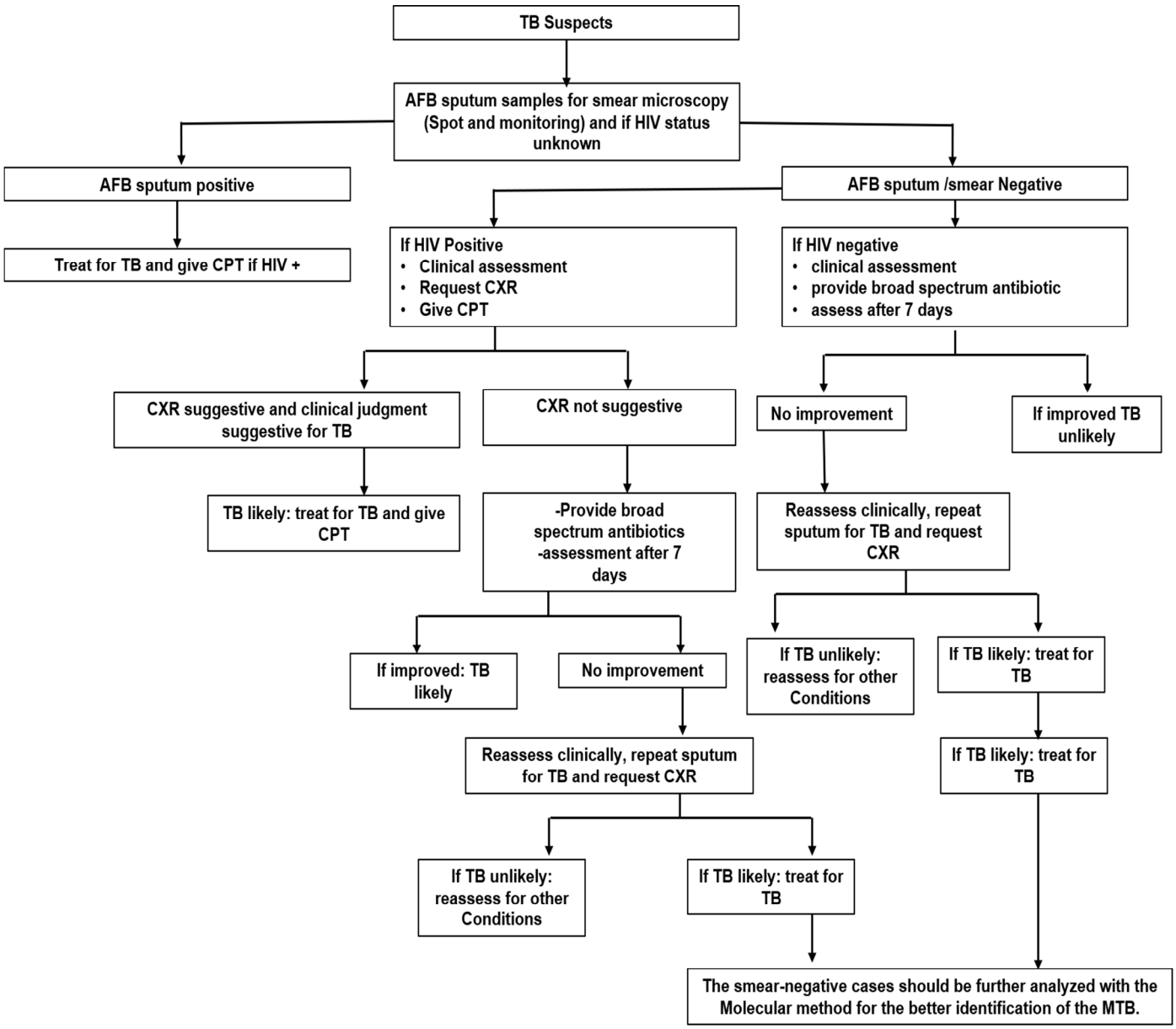

Fig 3: Flowchart on the diagnosis of pulmonary TB in children above 6 years/ adults and smear-negative detection and late EPTB: [75]

1.9 DIFFERENTIAL IDENTIFICATION AND DIAGNOSIS OF SMEAR-NEGATIVE DISEASE

As we know that the sputum samples are tested for the smear-negative or smear-positive identification. Positive smear test gives the PTB confirmation. Whereas, smear-negative cases are neglected which is leading to a major problem and also increase in TB cases in the Country? More focus toward smear-negative cases of TB infection should be laid in order to have a check over TB or EPTB. The smear-negative should be analyzed for the better identification of M. tuberculosis [51, 52]. One of the studies with the patient referred for sputum induction for about 15 months around 696 samples had suspected smear-negative or sputum-scarce TB[57]. About 62% (434 out of 696) and 74% (517 out of 696) of patient were referred for the elicition of TB with some HIV positive cases [58,59]. Alternatively, the sputum specimens collected may be inadequate in quality or number. Ipuge et al. (1996) found that 83.4% of smear-negative cases were detected on the first sputum collected sample, 12.2% on the second, and 4.4% on the third sample [92]. In Tanzania the studied revealed effective results on the Ziehl-Neelsen (Z-N) staining [60]. In US, the auramine-rhodamine stain was used in diagnostic laboratories and reported that 73% of the smear-negative cases were found on the first sputum collected sample, 14% on the second, 7% on the third, and 6% on the fourth etc., [60]. The study conducted in Trivandrum, South India (Santha et. al., 2003) revealed that out of 426 samples 63 were smear-positive, and of 293 were smear-negative. People with smear-negative cases express similar symptoms viz., coughing, sneezing, weight loss, high fever etc., but exhibit negative results in staining techniques [59]. The negative results should not be ignored as they may be positive with latent TB infection. Such cases should be further processed with molecular diagnostic approach for MTB infection [59].Many case studies have proved that around 25% of the smear-negative will be positive for the MTB. For instance, in the study of Hedwiga F Swai1 et al., (2011) 467 enrolled subjects. Of those, 318 (68.1%) were HIV positive, 127 (27.2%) had sputum culture positive for M. Tuberculosis, of which 66 (51.9%) correctly treated with anti-Tuberculosis drugs and 61 (48.1%) were missed with TB treatment. Of the 286 subjects with sputum culture-negative, 107 (37.4%) were positive treated with anti-tuberculosis drugs. The other diagnostic tool that reveals the patients with active TB and with only symptoms

of TB is another broad area. The presence of a dry cough, a high respiratory rate, a low eosinophil count, a mixed type of anemia and the presence of a cavity at lungs were found to be predictive of smear-negative but culture-positive pulmonary tuberculosis [60, 61]. From Alma Tostmann et al., (2008) study, it showed 394 clusters with a total of 1285 patients. Based on molecular linkage, 12.6% of the secondary cases were attributable to transmission from a patient with smear-negative TB. The relative transmission rate among patients with smear-negative TB, compared with patients with smear-positive TB, was 0.24% (95% confidence interval, 0.20–0.30). Secondary cases in clusters with an index patient with smear-negative TB more frequently had smear-negative status (odds ratio, 1.86; 95% confidence interval, 1.18–2.93), compared with secondary cases in clusters with an index patient with smear-positive TB. Conventional contact tracing revealed that 26 (6.2%) of the 417 sources, as identified by the Municipal Health Services, had smear-negative TB [62, 63]. Finally, smear staining may be technically inadequate for the identification and detection of the TB infection. The smear quality may be increased in some of the laboratories, hospitals and some research units especially in the HIV epidemic countries and states. So that the proper examination gives the better result in identifying the TB at the preliminary stage and TB eradication is achieved [64]. In Tanzania, compared the microscopic results with epidemiological study of the TB along with the HIV and smear-negative cases; they obtained about 29% of the new smear-negative cases based on the microscopic results. There exists a chance for false positive results which may result as smear-positive due to the inadequate staining techniques used in the laboratories, over decolorisation or inspection of two fields. ZN smear examined before the negative result of the smear which approximately takes 5-10 minutes [65]. If the smear is thick it can obscure the presence of AFB or may fail to give off the result. The patients with dual infection viz., HIV & TB, the diagnosis becomes very difficult due to fade immune system. Lucas et al., (1995) conducted a study of 247 HIV-positives along with nocardiosis and they found the nocardiosis nine times more with TB patients. ZN stain for AFB stain of sputum proved the worthwhile investigation of the negative results[65]. Kamanfu et al. (2007) found that a similar percentage (10.4%) of HIV-positive patients hospitalized with acute respiratory disease in Burundi had *Salmonella* bacteraemia, along with *S. Typhimurium*[66, 67]. Blood culture facilities are required to make this diagnosis but are rarely available [48].These two studies have shown gram-negative bacteremia which is considered in patients along with the pulmonary and smear-negative case study, particularly with the HIV-positive patients. These medical conditions account for significant morbidity and mortality in patients presenting with 'smear-negative pulmonary disease' in HIV- and TB endemic developing countries[68]. They concluded that bronchoscope is the best method for the smear-negative samples that gives better outcome [69, 70].

1.10 ADDRESSING THE PROBLEM AND TREATMENT OF SMEAR-NEGATIVE TB

There are many methods for treating smear-negative cases that optimize the detection in number of patients for the correct treatment of TB [71, 72]. In the study of Tanzania, 182 (Ipuge et. al., 1996) sputum-negative samples in which 41 are cultures were positive for TB [92]. The study revealed the presence of various diagnostic methods for related symptoms and that were associated with TB and 71% of the patients were associated with HIV-positive. Their study of symptoms included cough for 21 days, chest pain for 15 days, absence of expectoration, and absence of dyspnoea. The presence of any two symptoms from this can be further treated for the diagnosis TB with 85% sensitivity but only 67% specificity [73, 74]. If the three symptoms are present then the specificity improved to 86% and sensitivity reduced to 49%. From the study of Wilkinson et al., (1997) 237 African patients with TB suspects [78] of which 56 smear-negative were culture-positive. The smear-negative patients were treated with the short-term courses with ampicillin (500mg QID for 7-10 days)[76]. 50% of the smear-negative and culture-positive was finally diagnosed with a second line of drugs. Some patients also responded to the other chemotherapies and some for anti-TB drug trials. However, the remaining culture-positive patients appeared to respond to antibiotic therapy, either because of unrelated fluctuations in disease severity or successful treatment of superimposed bacterial infection and were incorrectly discharged. Furthermore, some culture-negative patients failed to respond to antibiotics or were misdiagnosed with TB. The specificity of the ZN staining treatment gives 84%. However, it has decreased to 78% when combined with antibiotic trials [77, 78]. In developing countries, some data is available for the prevalence of antibiotic resistance. At United States, the study included published (1996) data about the serotypes and resistance profiles of 5000 invasive isolates similar to the African continent studies for about 500 strains [79, 80]. The staining method also may give a false result, hence it's recommended for advanced methods for detection of TB viz., microscopic analysis, mycobacterial culture of tissue samples, nucleic acid-based testing, chest x-ray, tuberculin skin testing (TST) or interferon-gamma release assay (IGRA), etc.

2. CONCLUSION

The current scenario reveals the increased extra pulmonary tuberculosis cases worldwide. As per the records of RNTCP reports it is re-emerging in the country from 2017. Even though there are many drugs from the past 75 years, no suitable drug is available that diagnose the EPTB at primary stage. Even though the first line drug has around 99% accuracy, MTB still exists and is mainly because of the drug resistance. Over the period of the time of drugs prescribed the bacteria is gaining resistant towards that particular drug and are becoming more resistant. The smear-negative pulmonary TB is increasing the problems in the clinical sectors along with the HIV/TB epidemic. The case study detection can also be improved by managing and implementing many methods. The novel sputum processing techniques can be used widely by improving the investigation methods. Study revealed that culturing of sputum sample is heading to the new problems, because that lead to many disadvantages especially like time consumption, pathogen spreading, late growth, contamination of culture etc., Though there are many diagnostic methods such as AFB staining, TST, chest radiography, X-ray etc., drugs for treating the MTB are more expensive, new ideas to be included along with existing methods for testing MTB. The present work identifies the risk factor that supports considerable management aspects of extra pulmonary tuberculosis.

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5. AUTHORS CONTRIBUTION STATEMENT

Dr. Sumana K was initiated for this review articles and altered and edited the manuscript. Ms. TalluriRameshwari K R has finalized the topic and written the original Manuscript, Dr. Anuradha K suggested ideas for this review, and also helped in writing the manuscript order wise along with flow chart and diagrams etc. and Dr. Jayashree K and Dr. Raghuraj Singh Chouhan, has done with results interpretation, data collection, etc., Finally all authors have discussed the methodology, results and contributed to the final manuscript.

6. CONFLICT OF INTEREST

Conflict of interest declared none.

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