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REVIEW ARTICLE

A PATTERN OF TUBERCULOSIS INFECTION AN OVERVIEW

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ABSTRACT

Tuberculosis (TB) is caused by pathogenic bacteria Mycobacterium tuberculosis. It is an acid-fast bacillus (AFB), gram-positive, non-motile, aerobic organism. Each year millions of people became sick due to this disease. Beside this, it caused a huge number of deaths each year and hence is in the top 10 causes of death. In only 2007, 1.3 million people die due to TB. There are several diagnostic tools that can be employed to detect TB infection. This includes rapid molecular diagnostic tests, microscopic examination of sputum and culturing of an infectious agent. These methods have their own limitations and different strategies are employed to develop new tools. Besides this, different new approaches are urgently needed to develop efficient TB vaccine. Tuberculosis can be intervene at different points to develop efficient strategies. In this review, we try to summaries epidemiology of TB, different strains of *Mycobacterium tuberculosis*, vaccination for TB, different stages of Tuberculosis, intervening points, antimicrobial susceptibility testing and diagnostic strategies for TB.

INTRODUCTION

Tuberculosis (TB) is a bacterial infectious disease which remains one of the biggest health problems despite the fact that several control and preventive measures by the national and international community has been taken in the last decades (WHO, 2010). Tuberculosis (TB) is among the top 10 causes of death, each year millions of people became sick due to this disease alone (WHO, 2018). In 2017 alone it caused 1.3 million deaths and is responsible for 10 million new cases around the world. The World Health Organization (WHO) define Tuberculosis (TB) "as an infectious disease caused by the bacillus Mycobacterium tuberculosis which typically affects the lungs (pulmonary TB), but can also affect other sites (extra-pulmonary TB)". It is transmitted from a person to a healthy person by the expulsion of pathogenic bacteria by a person with pulmonary TB into the air like by coughing.

This review Paper is divided into three sections. The first section epidemiology. The second section describes available diagnostic tools for TB. While the third section highlights some of the risk factors for the transmission and development of TB.

Epidemiology: The causative agent of Tuberculosis disease (TB) is microorganism Mycobacterium. It is infectious in nature and generally infects the respiratory system in human. However, infection of other parts of the body can also take place. Fever, chronic cough, and weight are common signs of TB (WHO, 2018). TB is among the top 10 causes of death worldwide. In 2017, 1.6 million deaths were estimated due to TB.

There were cases in all countries and age groups, but overall 90% were adults (aged ≥15 years). Approximately one-third of the planet population is infected by TB (WHO, 2018). But, nearly everybody contamination by MTB is not infectious sometimes additionally 90–95% of contaminations do not show symptoms (Skolnik 2011). During 2012, a predictable 8.6 million constant occurrences had been actively found. During 2017, about 10 million fresh occurrences of Tuberculosis had been detected (Lozano et al., 2012, WHO, 2018).

China attained mainly remarkable development, through concerning an 80% decreases into their Tuberculosis death speed in the middle of 1990 and 2010. A figure of the fresh occurrences had been turned down with 17% in the middle of 2004 and 2014 (Kielstra 2014).

TB infection can be minimized by a screening of individuals with high risk, early detection of the disease and early treatment (Hawn et al., 2014; World Health Organization 2018). People with infection of TB also make other people in public, working place, home and social circle at high risk of infecting with TB. Although TB can be treated if timely diagnosed, with antibiotics but it requires a combination of several antibiotics which itself is hazardous to health and the environment. Besides this, an increasing number of resistivity to antibiotics make treatment of TB more difficult (WHO, 2018).

Vaccination: Extra-pulmonary tuberculosis and deaths among infants can be prevented by using Mycobacterium bovis bacille Calmette-Guérin (BCG) vaccine, however, there is no efficient vaccine which can prevent pulmonary TB in adults. This absence of an efficient vaccine makes TB one of the leading causes of death worldwide. Therefore,

new approaches for the development of an efficient vaccine against TB are of utmost importance.

Developing vaccine against TB is one of the primary considerations of WHO. Primary consideration of WHO is to develop vaccines against TB. In October 2017, the WHO assembled a meeting with global leaders in the TB vaccine advancement field to underline the WHO duty to this exertion and to encourage inventive ways to deal with the revelation and improvement of TB immunization applicants.

After so many decades while working in development of an effective vaccine for TB it is still not produced because of the nature of TB pathogen. The agent that causes TB is *Mycobacterium Tuberculosis* (MTB) that has several ways to maintains itself in host even for years and has structure and nature that makes it alive and resistant. It has unique machinery that help it to escape host immune response and help to adopt according to the host body to remain alive until it changes itself to cause the disease. Almost all components including proteins of envelope of MTB, the carbohydrates and lipids are major point of interest as they are subjected to the pathogenicity and virulence of the pathogen. There are several technologies that are used and have encouraging results these are DNA vaccines, lipid extracts, liposomes and membrane vesicle formulations (Sarmiento et al., 2019).

To prevent tuberculosis or likewise mycobacterial diseases a vaccine called Bacillus Calmette-Guerin (BCG) is developed and administered to humans first time in 1921. It is a live attenuated vaccine form of bacterium *Mycobacterium bovis*. The vaccine was developed by Calmette and Guerin. BCG is the only vaccine against tuberculosis and is regularly given to new-borns as immunization schedule and it gives protection against non-tuberculosis mycobacteria infections like leprosy and Buruli ulcer. Beside this, it also has used in superficial carcinoma of the bladder.

There are no fears of using BCG vaccine and it is safe to use. Like other vaccine it protects or specifically we can say prevents TB and other infections of mycobacteria (Fordham von Reyn and Vuola 2002). It is given to produce natural protection against tuberculosis infection (Edwards and Palmer 1968; Fine 1995). The immune response is generated because of mycobacterial agents. It is notable that after once disease occurred and cured it may reappear in future and recurrence may happen in both HIV Infected and HIV Non infected patients (Heimbeck 1948; Bjartveit 2003; Verver et al., 2005; Lahey et al., 2013; Zumla et al., 2013; von Reyn and Horsburgh 2006). It is also assumed that BCG decreased the child death rate in a non-tuberculosis cause. This decrease in childhood death rate could be because of epigenetic reprogramming of the NOD2 receptor (Roth et al., 2006; Kristensen et al., 2000; Kleinnijenhuis et al., 2012).

Common administration of BCG is done by either intracutaneously or intradermally. However now researches are focusing to administer the BCG through respiratory way as the naturally the disease occurs in humans through respiratory tract (Horvath et al., 2012; Jeyanathan, Heriazon, and Xing 2010).

Stages of Tuberculosis and Intervention Points:

The spectrum of Tuberculosis is generally correlates with the immune response of the host. A large number of infected people develop latent or persistent infection, however, only around 10 % people develop disease (Hanifa et al., 2009). These are supported by the fact that highly exposed people who are likely to be infected remain healthy. This suggests that there are some unknown immune mechanisms behind their protection from the diseases. Similarly, people with tubercle bacilli in their sputum lack characteristics of the disease despite the fact that they are able to transmit the infection (Bates et al., 2012; Mao et al., 2014**).**

Tuberculosis can be intervene at different points during the course of the disease. These include prevention of infection, prevention of latency establishment, prevention of conversion of latent phase into active phase and Treatment of an individual with active disease

Infection of most of the healthy people with *Mycobacterium tuberculosis* leads to no symptoms due to the fact that immune system of infected person kill or remove the pathogen by innate and acquired immunity. Cell mediated immunity in the infected person develops after 2-8 weeks of infection. In this granulomas remove the infection albeit it is only partial protection (Andrews et al., 2012; Chimusa et al., 2014)

Scientifically it is believed that infection does not lead to subsequent immunity against the pathogen. This is used to explain the occurrence of reinfection (Barry et al., 2009). Distinguish can be made between the bacteria responsible for prior infection and those new strain responsible for reinfection by employing molecular fingerprinting. The original infective strain can reestablish an active phase of the disease (relapse) after reemerging due to ineffective treatment (Khan et al., 2012). The different population have different rate of reactivation which range from 1 to 30 percent. It is unknown whether latent bacteria in infected people remain viable during life span or not, however, the chance of reactivation remains into old age (Stead and Dutt 1989).

Primary infection in some people on another hand results in active tuberculosis. Its symptoms include coughing (including sometime blood in sputum), dizziness, weight loss, pain in chest, fever and night sweats.

Tuberculosis is the outcome of a complex interaction between pathogen and immune response of infected individual which lead to successful replication of the bacteria and development of the disease (Atun et al., 2009; Shaler et al., 2013). Pulmonary TB is the main clinical appearance and accounts for 50-70 % cases while Extra-pulmonary TB accounts for 10-30 % cases (MacIntyre et al., 1997).

In the absence of other predisposing conditions, only 5–10 percent of infected people develop progressive primary disease within five years of infection (Hanifa et al., 2009). After five years, the annual risk of reactivation of latent infection is much lower. On the other hand if the infected individual is HIV positive this risk increase to10 %. However, the risk of progressing to active TB is age dependent. It is high in infancy became lower in relatively older children but increases at adolescent which continue to increase throughout adulthood (Hanifa et al., 2009; Isler et al., 2013).

Beside active TB latent TB also exist although the percentage of people with latent Tb is unknown. However it plays important role in the epidemiology and population dynamics of the disease. The people with latent TB is a big reservoir of potential disease and transmission to other. Beside this, a long time latent infection also provide certain degree of protection against TB (Andrews et al., 2012).

***M. tuberculosis* Strains:** Different evidence suggest that evolution of the main strains of Mtb is linked with major human migrations from Africa to other continents such as America, Europe and Asia (Gagneux 2012). Although early studies suggest minimal diversity among these strains (Keane et al., 2001) but further genomic studies showed more variation (Alhajri et al., 2011). Later studies increase the understanding of Mtb strains and their global transmission (Talat et al., 2010; Hayward et al., 2018). Beside this, other investigators (Cegielski, Arab, and Cornoni-Huntley 2012) tray to reveal whether variation between or within strains contributes to the pathogens ability to infect hosts or to the natural history of the diseases (Alhajri et al., 2011; Wilkinson et al., 2000). As different studies continue some showed the association of variation in Mtb strains with the probability of spreading tuberculosis among household exposures (López et al., 2003; Saelens, Viswanathan, and Tobin 2019; Liu et al., 2018)

Different studies revealed that Mtb strains vary in their strength and mechanism of inducing host immune system (Baker et al., 2012), competitive ability within host (Boelaert et al., 2007), mutations acquiring (Den Boon et al., 2016) and developing drug resistance (Borrell and Gagneux 2011; Advani et al., 2019). All of these can potentially affect progression of infection, disease and response to particular therapy (Gagneux 2012).

Overall, accumulation of mutations decreases the fitness of pathogens. Evidence suggest that strains are different in their ability of transmission in human population and animal models. So it is possible that a new mutation may change the fitness of the pathogen. Despite this mathematical models were also employed for strain research (Niewiadomska et al., 2019) such as emergence of drug resistance where it shows that strain diversity may contribute to it (Basu and Galvani 2008).

Antimicrobial Susceptibility Testing: Most of the infectious diseases are caused by pathogenic bacteria. To provide proper treatment to the infected individuals it's important to determine the resistivity and sensitivity of infection causing pathogen to different antimicrobial agents by growing in labs using growth medium called culture (Lagier et al., 2015; Graham et al., 1985). Antimicrobial susceptibility testing (AST) is a technique that is used to determine which antimicrobials are productive for specific persons. Conclusively it is the thing that is used in healthcare centres to treat patients and prevent the infectious diseases. As currently, bacterial mutations are occurring more frequently, it is of utmost importance to have a close eye on the resistance patterns of pathogenic bacteria including *Mycobacterium tuberculosis* (Sawatzky et al., 2015; Graham et al., 1985). Nowadays several techniques are available commercially and developed in laboratories to check the susceptibility and resistivity of a particular or group of bacteria. These also include disk diffusion and minimum inhibitory concentration (MIC) methods. Regular bacterial resistivity tests such as those for Gram positive (e.g., *Staphylococcus aureus*) and Gram negative (e.g., *Pseudomonas aeruginosa*) are usually performed in common laboratories while for Mycobacterium tuberculosis it is performed in specialized laboratories. Regardless of the method employed for resistivity check of bacteria, it is important for the laboratories to be very analytical in order to avoid pseudo results and obtain right results as it will affect the treatment or elimination process of the pathogenic bacteria.

Sampling for both disk diffusion and MIC techniques are as same as any other sampling is it also requires growing of colonies on particular culture

media. For susceptibility test different type of samples are used in laboratories it generally includes urine, wound, stool, sputum, body discharges, blood and cerebrospinal fluid (Coorevits et al., 2015).

With the advent of the technologies it is now a days not necessary to grow colonies for the susceptibility test as some commercial labs use techniques that directly determine the resistivity by detecting the resistant genes. Example of such a technique is GeneXpert MTB/Rif. In this technique susceptibility or resistivity of *Mycobacterium tuberculosis* towards rifampicin is detected directly by only subjecting samples such as sputum to the automated machine (Xie et al., 2017; Feyisa et al., 2019).

It is important to test the susceptibility of antimicrobials in patient who get infection from some specific pathogens. Despite this review is all about Mycobacterium tuberculosis but it is necessary to point out that it is possible to determine susceptibility of antifungals towards fungal infections (e.g., Candida, Aspergillus spp). Not only that also antiviral susceptibility test are also available to acknowledge viral infections (e.g. influenza virus) using molecular based techniques which may include analysis of sequences of genes like Sanger and pyrosequencing methods (Santos et al., 2006).

For antimicrobial susceptibility test (AST) to be effective, it is necessary to identify and diagnosis the pathogenic agent itself that cause the disease. Because two patients may not be treated similarly even, they show similar signs and symptoms because the causative agent may have different strains which are differently susceptible to different drugs. For example, two patients infected with *S. aureus* may have one with methicillin resistant strain and while other with ordinary strain. Similarly, two patients effected by *Mycobacterium tuberculosis* may have one with multi-drug resistant strain and other with drug-susceptible. So it is necessary to diagnose and identify the causative agent along with susceptibility or resistivity test.

Results of AST are influenced by certain factors such as sample collection, test procedures and even reporting. A small confusion or change from specific standard AST procedures may lead to significant change in results and which may also affect patient diagnosis and his treatment. Even the way sample is collected also affects the outcome of the test. Incorrectly collected samples may lead to false or inappropriate result. Hence it is important that person performing the AST procedures should be well trained and quality control should be strictly applied to the procedure. Despite the expertise of operating person, there are also some other factors that influence the result of AST. These include the quality of using apparatuses and strict adherence to personnel protective equipment as it may otherwise increase the chance of contaminating laboratory equipment.

Diagnostic strategies for TB: There are several diagnostic tests available for TB, however, they have specific limitations. Investigation has been done for detecting diagnostic biomarkers for TB (Goletti et al., 2016; Weiner and Kaufmann 2017; Wallis et al., 2009) as well as predictive markers as disease progresses from latent to active form (Petruccioli et al., 2016). Latent TB should be a focused immediately as it converts sterile immunity to non-progressive disease which certainly becomes active TB (Barry et al., 2009; Salgame et al., 2015). So far the present diagnostic test fail to differentiate between latent and Active TB (Pai et al., 2014; Auguste et al., 2017; Nemes et al., 2017). Active TB is diagnosed by making sure the presence of *M. tuberculosis* such as by performing acid fast staining or direct cultivation of bacteria from sputum samples. On one hand Microscopy is faster while on another it is insensitive to detect the pathogen. Likewise growing the pathogen in culture is more sensitive while media requires at least 6-8 weeks or may be more to detect the pathogen making it much slower for timely detection of TB. Beside this Standard liquid culture such as Gene Xpert MTB/RIF and Xpert Ultra are newly used techniques.

Another test used for the diagnoses of latent TB is tuberculin skin test (TST) or the interferon-γ release assays (IGRAs). In TST Purified tuberculin derivative (PPD) are utilized which are nonspecific to *M tuberculosis*. However, TST has the limitation that it gives pseudo positive results when subjected to individuals with exposer to ubiquitous non-TB mycobacteria or those having BCG vaccination. Beside this, several others test like IGRAs and T-spot are available in the market. These tests such as TST and IGRAS have limitation that they only help to manage the patients and none of them predicts that progression of latent TB to active TB also. In addition, it doesn't differentiate if the response is because of present infection or old memory of immune cells (Pai et al., 2014; Auguste et al., 2017; Nemes et al., 2017; Mahomed et al., 2011; Banaei and Pai 2017).

Only an ideal test can differentiate between active TB and latent TB and the cured one and for this ideal test ideal sample could be the human urine sample. A case of another technique that has potential to be used for both latent as well as active TB depends on the assurance of specific circling B lymphocytes in the peripheral blood (Sebina et al. 2014). It has been demonstrated that B lymphocy-

tes may separate people with active and latent TB (Lu et al., 2016).

Further, Blood RNA signature with sensitivity of 53.7% and specificity of 82.8% can identify risk of progression to active TB (Zak et al., 2016). The same has been also found in several studies of African countries. The Finding of these studies strongly argue for the potential of using RNA signature as a tool to validate the risk of progression towards active TB.

Metabolic products could also be used as a biomarker for the diagnosis of TB but most of these metabolites of human's body lack structural information (Mahapatra et al., 2014). But an excited exception is N1-acetylisoputreanine which is novel polyamine metabolite in human urine. This metabolite has the potential to be used as biomarker for the detection of *M. tuberculosis* infection (Fitzgerald et al., 2017).

In one particular study to look for biomarkers for pulmonary TB in urine specimen, it came in notice that 10 mycobacterial related proteins were present as special biomarkers for active TB and 6 set of different proteins were of latent TB. However, their partial identification is achieved so far (Young et al., 2014).

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