IMPACT OF EARLY DIAGNOSIS AND MONITORING ON TUBERCULOSIS PROGRESSION

Dissertation Submitted to

The IIHMR University, Jaipur

for the partial fulfilment for the award of the degree of

Master of Business Administration (MBA)

in

Pharmaceutical Management

By

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Under the Supervision and Guidance of

Dr. Sudhinder Singh Chowhan

2024

IIHMR UNIVERSITY

CERTIFICATE

This is to certify that **Ms. Rakshita Karambelkar** in partial fulfillment of the requirements for the award of the degree of MBA Pharmaceutical Management) from the IIHMR University, Jaipur has successfully completed her internship at (IIHMR University) during March 14 - June 13, 2024.

01/07/24

Place: Jaipur
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DECLARATION BY STUDENT

I Rakshita Karambelkar Enrollment no. IIHMR-U/02/2022-24/0423 hereby declare that this dissertation titled STUDY THE IMAPCT OF EARLY DIAGNOSIS AND MONITORING ON TUBERCULOSIS PROGRESSION is the bonafide record of my original research work. I declare that it has not been submitted to any other university or institution for the award of any degree or diploma. Information derived from the published or unpublished work of others has been duly acknowledged in the text.

Name of Student: Rakshita Karambelkar

Date – 02|07|2024

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CERTIFICATE

This is to certify that dissertation titled **"The impact of early diagnosis and monitoring on tuberculosis progression"** is a record of the research work undertaken by **Ms. Rakshita Karambelkar** in partial fulfillment of the requirements for the award of the degree of MBA (Pharmaceutical Management) from the IIHMR University, Jaipur under my guidance and supervision and herapproach to the research study has been sincere, scientific, and analytical.

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ABSTRACT

Introduction:- Tuberculosis (TB) remains a major global health challenge which is a highly infectious disease caused by Mycobacterium tuberculosis and was discovered by Robert kosh in 1882, with an estimated 10.6 million new cases and 1.6 million deaths worldwide in 2021. Early diagnosis and monitoring of TB progression are crucial for improving patient outcomes, reducing disease transmission, and achieving global TB control targets. World Health Organization (WHO) developed the End TB strategy with a goal to end tuberculosis epidemic by year 2035. This thesis aims to systematically review the impact of early diagnosis and monitoring on the progression of TB, from latent infection to active disease. It examines the various stages of TB infection, including eliminated TB, latent TB infection, incipient TB, subclinical TB, and active TB disease. Various diagnostic methods are used for detecting tuberculosis which are very helpful in providing appropriate diagnosing and treatment of the disease.

Objective:- The objective is to study the impact of early diagnosis and monitoring of tuberculosis progression by using various diagnosis methods and the limitations of conventional methods.

Methodology:- The methods used for the diagnosis of tuberculosis detection are chest radiography, tuberculin skin test, sputum smear microscopy, loop mediated isothermal amplification, line probe assay. The new methods used are digital PCR, CRISPR/cas 12a, gold nanoparticle, Au mediated dipstick, micro RNA which will help in proper andearly diagnosis of this infection.

Key result:- The result of the study conduct shows that advanced diagnostic methods are very helpful in determining tuberculosis as they are not time consuming which results in the proper and timely diagnosis of the infection. In addition to that we also studied the impact of early diagnosis and monitoring on tuberculosis.

Conclusion:- This study shows that diagnosing and monitoring TB early is crucial for improving patient outcomes, reducing the spread of the disease. We also learnt about various new diagnosis methods which help in enhancing tuberculosis detection.

Key Words:- Conventional methods, digital PCR, latent TB, incipient TB, subclinicalTB, mycobacterium tuberculosis.

ACKNOWLEDGEMENT:-

It gives me immense pleasure to acknowledge and thank all those who have given consistent guidance, advice and encouragement without the support and guidance it was not possible for me to complete this task.

I take this opportunity to express my deep, sincere gratitude and thanks to my academic professor DR. SUDHINDER SINGH CHOWHAN, IIHMR University for their guidance and support throughout the dissertation.

Moreover, I would like show my heartfelt gratitude to the respondents who gave me their valuable time during my dissertation, inspite of their busy schedule and helped me complete my dissertation.

Lastly, I am much obliged to my family members and friends for their love, concern, encouragement and moral support.

Rakshita Karambelkar

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CHAPTER 1: INTRODUCTION

Tuberculosis (TB) is a highly infectious disease caused by the acid fast pathogen Mycobacterium tuberculosis (Mtb) and predominantly infect the lungs and was discovered in 1882 by Robert Koch. But it also attacks other important organs of the body causing extra-pulmonary TB. Mtb transmission occurs commonly via air droplets (produced by coughing, sneezing, etc.), as tuberculosis is a communicable disease. According to global tuberculosis facts given by WHO 2022, Millions of people are getting ill from this disease each year that is nearly around 10.6 million and almost 1.6 million people of the world die from TB (including people that have become HIV-positive). In 2020, there were roughly 5.8 million cases of newly identified cases of tuberculosis (TB), down from 7.1 million in 2019. We therefore need advanced TB diagnostic methods like this that are leader and more likely and cause no barrier for other pandemics in the future.

TB patients were predominantly adults (> 90%), more cases appeared in men than in females. Worldwide, one in four individuals show an immune in response to a Mtb infection, and they possess the possibility for transforming into an active illness or stay latent. It was previously referred to as latent tuberculosis. Pulmonary tuberculosis (PTB) is a synonym for tuberculosis that affects the lungs; extra-pulmonary tuberculosis (EPTB) is the term for tuberculosis that affects other organs.

Tuberculosis (TB) is a major worldwide killer with an annual death toll of at least 1.2 million people. Preventing infection and mortality among invisible patients whom the existing detection methods have not been successful to detect.The immunological response to tuberculosis (TB) is an ongoing process, and recognizing individuals who have gone transition from "latent tuberculosis infection (LTBI)" to active TB disease is almost as important as determining whether patients require ultra-short treatment durations.

Due to T cell reactivity to TB antigens, tests like "Interferon-gamma release assays (IGRAs)" can suggest that a person was exposed to Mtb. However, because of their low sensitivity and specificity, antibody tests are not recognized by the WHO for the diagnosis of tuberculosis. The incapacity of current diagnostic techniques to

differentiate between latent infection and active tuberculosis disease, high infrastructure requirements, low sensitivity, lengthy turnaround times, and expensive are only a few of their drawbacks. Patient treatment is currently being improved by molecular diagnostic tests, which offer a quick TB diagnostic platform with results in under two hours. The Gene Xpert test is currently routinely used for TB illness screening in high-TB endemic settings in settings with low resources. For both drug-sensitive and drug-resistant TB, the right treatment must be administered based on an accurate diagnosis of TB. For six months, isoniazid (H), rifampicin (R), pyrazinamide (Z), and ethambutol (E) are the drugs prescribed for drug-sensitive tuberculosis. Drug-resistant tuberculosis is caused by resistance to any of these medications; this condition can be managed with a mixture of second-line medications, including bedaquiline, cycloserine, clofazimine, delamanid, and fluoroquinolones (levofloxacin or moxifloxacin). Since 2007, tuberculosis (TB) has become the leading cause of death globally and the leading infectious illness. The Global Tuberculosis Report 2021 states that 9.87 million people globally suffer from tuberculosis (TB), with India, China, and Russia bearing the greatest burden of the disease with approximately 2 billion cases of MTB [11]. To achieve the complete eradication of tuberculosis, the World Health Organization (WHO) developed the End TB Strategy. The 67th World Health Assembly approved the strategy in 2014 with the goal of ending the tuberculosis epidemic worldwide by 2035 (WHO, 2021). In the beginning the plan was to reduce the number of people with tuberculosis by 90%, as well as the death rate by 95% and protect families from the negative effects of tuberculosis. The primary methods to accomplish this goal include stepping up efforts to detect and treat cases of active tuberculosis, screening everyone at high risk, and providing preventive medicine to those who are at risk of contracting the disease.

1.1 PROGRESSION OF TUBERCULOSIS INFECTION:-

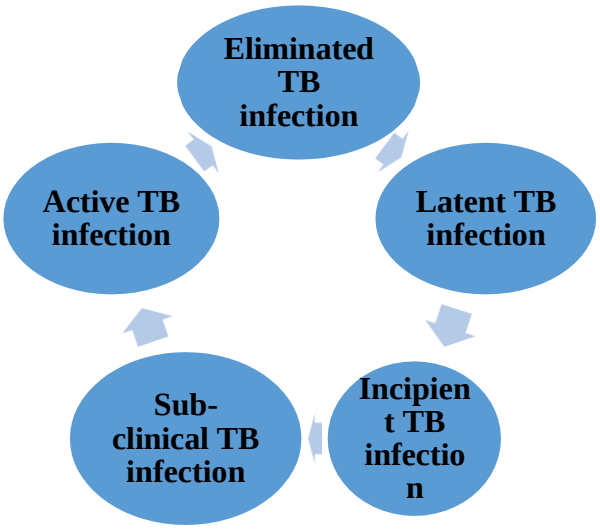


Fig 1:- Flowchart showing progression of tuberculosis infection.

Eliminated TB infection:- A person with an eliminated tuberculosis infection has previously been exposed to M. tuberculosis; they have either overcome the disease by innate and/or acquired immune responses, or they have been cured of the infection with anti-TB drugs. Although the M. tuberculosis bacteria in this person are no longer alive, there may still be immunological signs of a previous infection.

Latent TB infection:- The term used to describe tuberculosis infection in the past was latent tuberculosis infection (LTBI). When there is no clinically active tuberculosis, this phrase has been applied to characterize a state of persistent immune response to stimulation by Mtb antigens using assays such as the interferon (IFN)-γ release assay (IGRA) or the tuberculin skin test (TST).

Incipient TB infection:- The most challenging to characterize is intermittent TB, which falls between TB infection and subclinical TB due to the incredibly little evidence. The bacilli are living and most likely alternate between modest metabolic activity and replication intervals and periods of hibernation, which is characteristic of the TB infection state. Most likely, the patient is not contagious. The diagnosis of incipient tuberculosis lacks proven instruments. The results of the infection diagnostic tests (TST and IGRAs) are positive and stay positive as the TB disease progresses. Future research may uncover host ribonucleic acid (RNA) signals that could lead to the development of novel biomarkers that will allow for the precise diagnosis of incipient TB that will progress to subclinical or clinical TB.

Subclinical TB:- was described as a "disease" caused by viable Mtb bacteria that causes various abnormalities that may be found using current microbiological or radiological techniques but does not produce clinical TB-related symptoms.

From the TB infection stage (dormancy, no abnormalities, no inclination to advance towards the following stages) to the incipient TB stage, it symbolizes an evolution whereby bacilli may replicate intermittently and potentially evolve towards the next stages without any abnormalities being detected. The standard clinical criteria are not very helpful because symptoms might either be present or absent (even if the patientis unable to recognize them). As a result, although potentially contagious, the patients at the subclinical stage either have no symptoms at all or very few that they are unable to recognize.

Active TB:- Infection is caused by a live strain of M. tuberculosis and shows as clinical symptoms, abnormal radiographs, or microbiologic evidence of active tuberculosis. As of right now, the World Health Organization defines "symptomatic patients with radiological or microbiological evidence of M. tuberculosis" as having active TB disease. This classification would remain consistent with that.

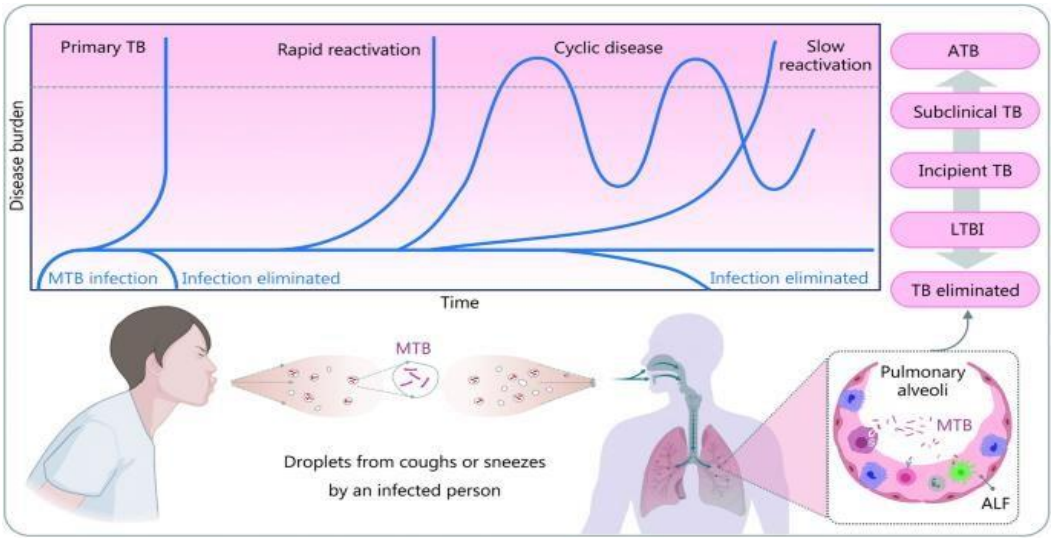


Fig 2:- This figure depicts the progression of tuberculosis infection from latent TB to active TB.

1.2 PATHOGENESIS:-

Mycobacterium tuberculosis is spread by inhaling suspended aerosolized air droplets, which have a size range of 0.65 to 7.0 μm, from an infected individual to a healthy one. When a healthy individual inhales these droplets, the germs pass through the trachea or

the nasopharyngeal area before adhering to the alveolar macrophages' surface in the lungs. Eventually, the germs are ingested by the macrophages in a process known as phagocytosis, which produces a phagosome. The pathogen recognition receptors that the mycobacterial conserved molecular patterns engage with toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors, and C-type lectins on macrophages to trigger an innate immune response against Mtb. As the primary defense cell against invading infections, macrophages are responsible for the generation of reactive oxygen species (ROS), hydrolytic enzymes, and acidity of the imprisoned bacterium. A phagolysosome, with a pH of 4.5 to 5, is created after the phagosome grows and combines with the lysosome. Acidification is mediated by this process. As soon as the acidification process begins, reactive oxygen species (ROS) and reactive nitrogen species (RNS) both reduce the metabolism of the microorganism. likewise macrophages spread copper and zinc, which are more detrimental to Mtb. In this adverse setting, the components of the bacterial cell wall are stimulated to lyse, killing the phagocytosed bacteria. The bacilli that sustain under such harsh circumstances grow and divide inside the phagosomes, increasing the bacterial burden within the host and resulting in the production of cytokines. Chemokines are a family of small molecules that are generated by Mtb-infected macrophages that aid in the management of the infection. The immune3Q cell trafficking that initiates the T cell response and draws monocytes, neutrophils, and dendritic cells to the infection site is governed by all of these inflammatory substances. The secretory proteins produced by Mtb are then processed by dendritic cells (DC). They are necessary for the antigens to be shown.

The development of a structure called a granuloma—which calcifies to enclose the immune system's bacilli, or Gohn complex—occurs with the commencement of the adaptive immunological response. Granuloma development, a stage at which the immune system halts the disease's progression, is frequently recognized as the signature of tuberculosis infection. The bacteria's transition into a stage that is not multiplying and has very little metabolic activity, known as the dormant stage, is a characteristic of latent tuberculosis infection. The process of resuscitation, or the restoration of the metabolic and replicative processes, is what causes the dormant bacteria to develop again. According to research, there may be a small number of metabolically active bacteria called scouts in addition to nonreplicating microorganisms. The scouts alert the other hibernating bacteria to bring back when the conditions are right. Subsequently, the active bacteria proliferate and multiply, leading to granuloma caseation and the release

of active Mtb to a different location, like lymph nodes or adipocytes, where it can avoid being recognized by the immune system. The host becomes coughy as a result, and Mtb bacilli are released into the air as aerosols (active TB infection).

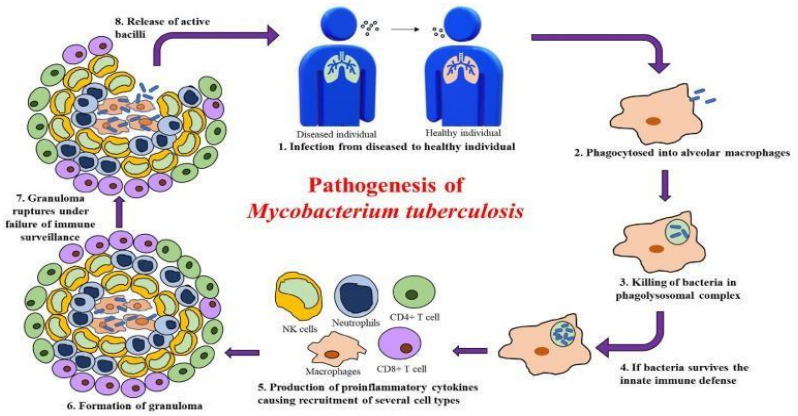
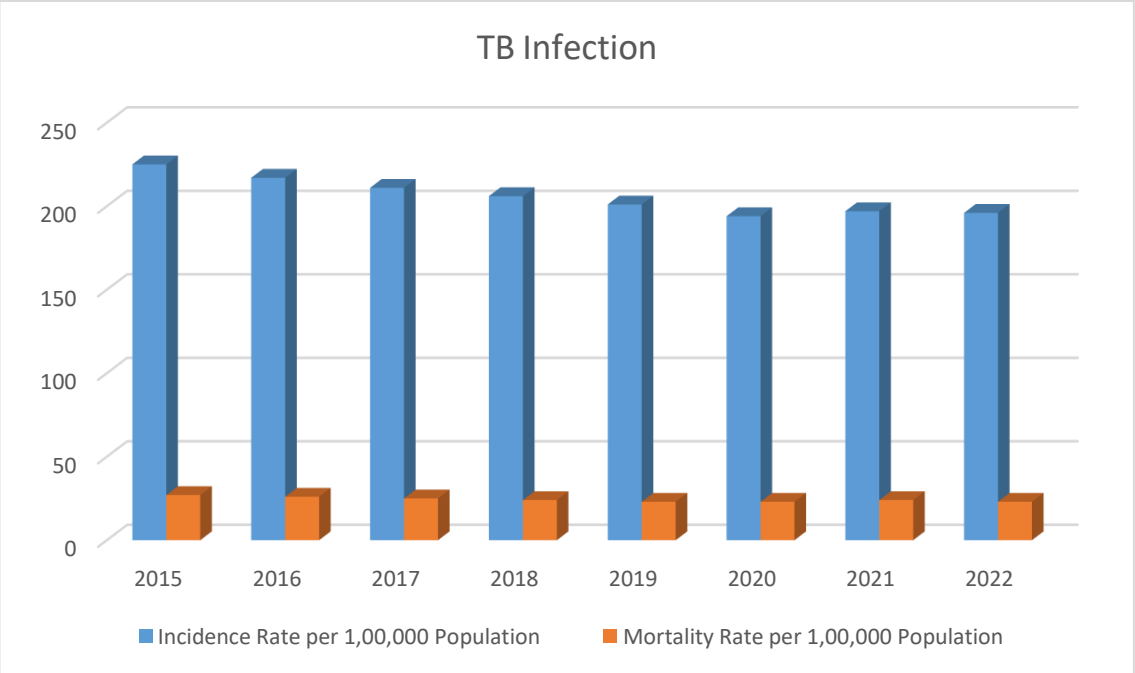


Fig 3:- Pathogenesis of Mycobacterium tuberculosis.

S. NO.	YEAR	Incidence rate per 100000 population	Mortality rate per 100000 population
1.	2015	225	27
2.	2016	217	26
3.	2017	211	25
4.	2018	206	24
5.	2019	201	23
6.	2020	194	23
7.	2021	197	24
8.	2022	196	23

Table 1: Estimated incidence and mortality rate per 100,000 population.



1.3 DIAGNOSIS OF TUBERCULOSIS:-

Premature death and mortality by tuberculosis can be minimized with early diagnosis and identification. The growing number of patients who are resistant to drugs poses a threat to the goal of eliminating tuberculosis. The ability to identify infections using laboratory diagnostic procedures has advanced tremendously. These strategies either take the place of or add to the present standard methods. Modern methods include CRISPR Cas, Gene Xpert, and LAMP, while traditional methods include microbiological culture, Acid-fast Bacillus smear microscopy, and others. Although traditional methods are slow and difficult, they are surprisingly common in countries where tuberculosis is common. Therefore, a correct diagnosis of tuberculosis is essential for reducing the complications.

1.3.1 Conventional methods:-Microscopy, which uses a microscope to see live bacilli, and radiography, which examines a chest X-ray to detect lesions, are two different techniques used to diagnose tuberculosis early. Latent tuberculosis infections are diagnosed by ELISA, IGRA, and the tuberculin skin test. The tests are based on the allergic reaction that the host produces in the presence of the Mtb infection. To detect multi-drug resistant tuberculosis, some of the most accurate and specific assays that need to be employed include RT-PCR, DNA microarray, and LAMP. The methodsare as implies:-

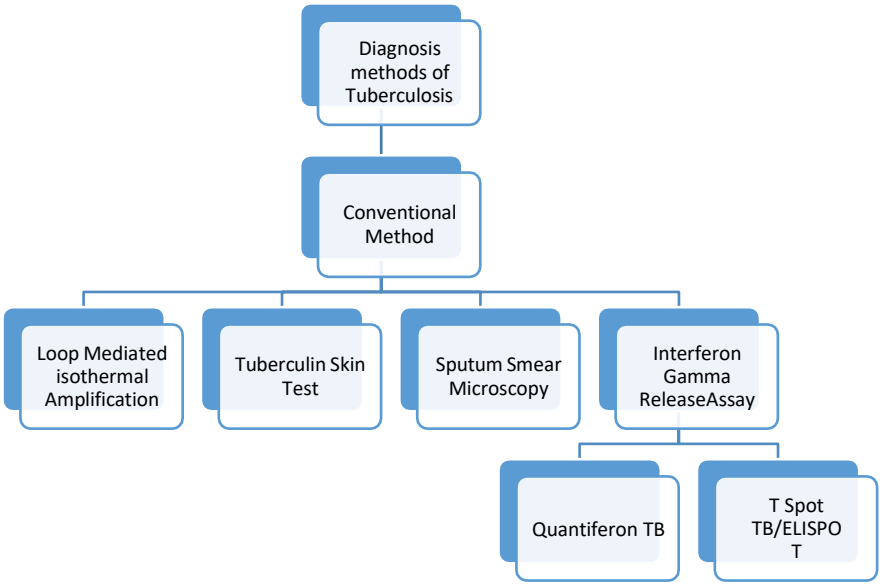


Fig 4:- Flowchart showing conventional methods of tuberculosis

Tuberculin Skin Test:- The tuberculin skin test (TST) is a well-known illustration of the hypersensitive cellular T cell response that takes place after mycobacterial antigen inoculation. It is based on a belief that, due to the presence of bacterial protein, a person exposed to the antigen will surely display an immune response. Purified Protein Derivative (PDD), the most popular Mtb antigen, is administered topically to the patient. The patient develops edema, fibrin deposition, and redness 48–72 hours after the injection as a consequence of T cells migrating to the injection site, where they release neutrophils, monocytes, and lymphokines. TST, the method commonly employed to diagnose tuberculosis, has a number of serious flaws. The main basis for concern with TST is its lack of specificity. False-positive results can occur as the test cannot differentiate between Mtb infection and non-tuberculosis mycobacteria or immunization with Bacille Calmett-Guérin (BCG). Both kinds of false positive responses typically occur by cross reactivity, which occurs when homologous antigens from ambient non-pathogenic mycobacteria or the BCG vaccine produce a reaction in the immune system. Considering these problems, TST remains the most used platform for diagnosing Mtb infection.

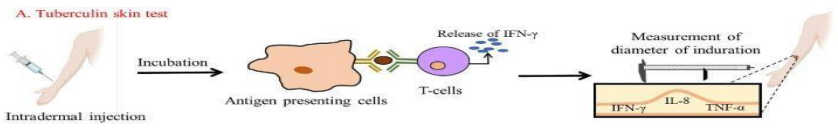


Fig 5:- Detection of Latent tuberculosis infection.

Interferon Gamma Release assay (IGRA):- If the patient is not responsive to the aforementioned antigens, ESX-1 substrate protein C (EspC) may serve as a good substitute. Since IFN-γ production is a crucial biomarker for tuberculosis infection and for determining a patient's response to treatment, it can be directly correlated with the bacillary burden or antigenic load. The assay is based on the patient's immune response to Mtb in an immunological-competent person. After being exposed to the pathogen, the memory T cells become activated. They then go on to digest the antigenicpeptides, preparing the memory T cells for the possibility of a relapse or reinfection.
These T cells release lymphokines, mainly interferon-gamma (IFN-γ), in reaction to stimulation by antigens specific to the Mtb complex, such as the Culture Filtrate Protein (CFP-10) and Early Secretory Antigen Targeting 6 (ESAT-6), that circulate in the blood during Mtb infection. There has been a strong T-cell response to these two. These two thus constitute the most often used targets for the Interferon-gamma Release Assay. The Quanti FERON-TB Gold test and the ELISPOT assay are the two different types of Interferon Gamma Release Assays.

a.) Quanti FERON-TB:- The amount of IFNγ produced in reaction to mycobacterial antigens such as ESAT-6 and CFP 10 given to a blood sample is determined using a quantitative assay termed Gold. While these antigens are recognized as epitopes by CD4+ and CD8+ T cells, CD4+ T cells are mostly activated to release interferons. The suspected patient's blood is extracted, and the aforementioned antigens are incubated with it for the whole night at 37 °C. The cells become sensitized to mycobacterial antigens and emit IFN-γ, which can be tested to aid in the diagnosis of tuberculosis. It is very specific and fairly priced for children aged 1 to 15.

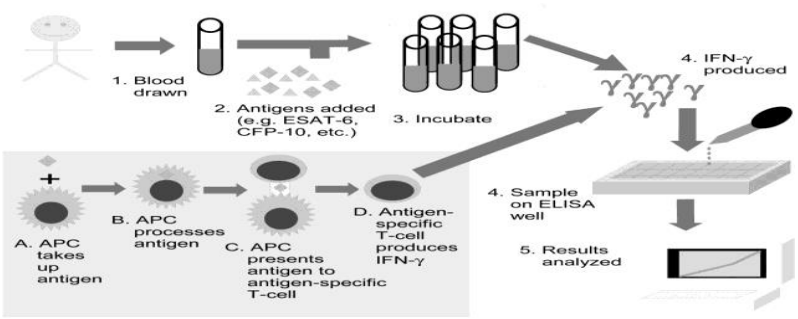


FIG 6:- Detection of tuberculosis with Quanti FERON-TB

b.) T spot TB/ELISPOT ASSAY:- The test determines whether a person has a Mtb infection by measuring the number of cytokine-producing T cells in the body. The

suspected patient's blood is used to separate the mononuclear T cells. Antigenic peptides such as CFP-10 and ESAT-6 are sown into a microwell holding the cell solution. Following an hour-long incubation at 37°C and cleaning in phosphate buffer, the microwells are inspected under a light source. Each dot on the well indicates a cytokine-producing T cell. Spot count and tuberculosis infection have a close correlation. A high spot number denotes an active TB infection, while a low spot number suggests a latent illness. Though there are number of benefits, like a decrease in false-negative results despite its great suitability for high-risk individuals, this test is not sensitive to various TB infection subtypes.

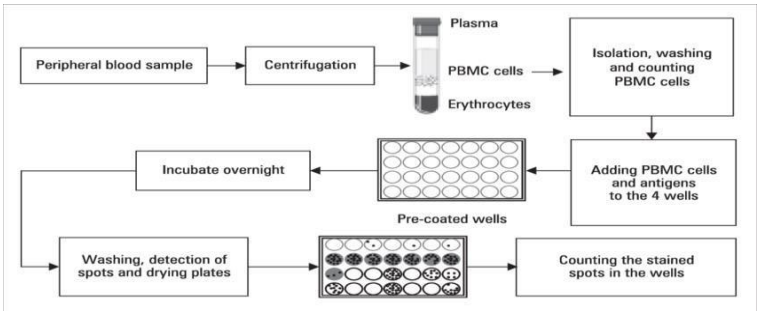


FIG 7: Detection of tuberculosis with T spot TB/ELISPOT ASSAY

Sputum Smear Microscopy:- The gold standard for diagnosing tuberculosis is thought to be cultivating Mtb in an appropriate growth medium and then examining the sample under a microscope. To isolate the bacterium, sputum samples or laryngeal swabs are taken from the suspected patients. Later that, the specimen is cleaned with a mucolytic agent made of 7% saline and 4% NaOH. The harmful fatty acids cannot attach to the mycobacterial cell wall in the presence of albumin and inorganic ions. Centrifugation of sputum in a solution comprising sodium hypochlorite and ammonium hydroxide can concentrate it, enhancing sensitivity and enabling quick identification of the bacillus. Staining necessitates the high lipid content of Mtb's cell wall to be able to tolerate acidic conditions. The staining method that is frequently employed is the Ziehl-Neelsen procedure. One other approach is the cold staining technique. Both of these include the use of basic dyes such as fuschia (primary dye), methylene blue (counterstain), and phenol chemicals, which puncture the bacterial cell wall. The acid-fast bacteria appear red under a microscope because they can absorb the blue color of the counter stain but not the red color of the primary dye. In order to enhance the detection of light- emitting diodes, fluorescence microscopy is often recommended.

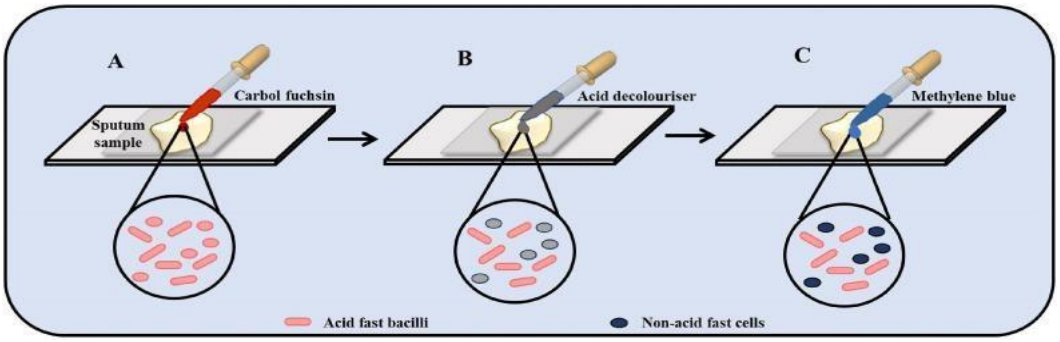


Fig:- 8 Microbiological observation of Mtb by acid-fast staining.

Loop Mediated Isothermal Amplification:- One method for identifying the genes causing multidrug resistance in Mtb is called LAMP. This is a new colorimetric technique that uses mycobacterial DNA amplification. By using this method, the genes causing resistance to various anti-TB medications, including ciprofloxacin, amikacin, rifampicin, and isoniazid, can be identified. This method focuses on the hotspot regions of the genes causing the mutation, as these are frequently the main causes of point mutations in these genes that lead to multidrug resistance [35]. Primers (allele-specific primers) called the forward (FIP) and backward (BIP) are made especially for these genes, which bind to the region that determines antibiotic resistance, and are used in the first step. The primers must fully anneal to the target sequences in order for the DNA strands to be amplified. The loop primer elongation then displaces the resultant product. As a result, the substitute product is amplified and has a biotinylated LAMP attached. The biotinylated LAMP product is elongated and displaced by the allele-specific primers. Allele-specific primers are then used to extend the biotinylated product. By combining primers specific to the mutated allele with a 60% specificity with LAMP primers, multiple additional mutations can be identified concurrently. Because it is affordable and doesn't need a complex platform, the peripheral health care system canuse it [36].

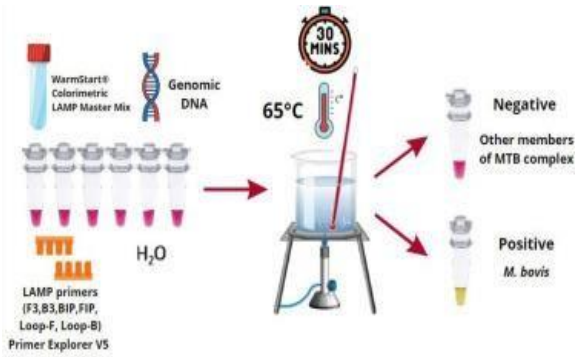


Fig 9:- Loop mediated isothermal amplification test for detecting tuberculosis.

Table 2:- Showing Limitation of conventional methods for tuberculosis diagnosis.

S. No	Conventional method	Description	Limitation
1.)	**Tuberculin Skin Test**	Tuberculin skin test is atype of test in which a person is exposed to antigen and shows immune response if bacterial protein is present.	The basic concern with TST is lack of specificity. The test often gives false-positive results because of the inability to differentiate between *Mtb* infection with that of non-tuberculosis mycobacteria or vaccination with BacilleCalmett-Guérin (BCG)
2.)	**Loop Mediated Isothermal Amplification(LAMP)**	**This method is used for identifying the genes causing multidrug resistance in Mtb iscalled LAMP**	The limitation of this assay is its complex reaction scheme and primer designing, whichcan be complicated, especially for new users.
3.)	**Sputum Smear Microscopy**	**It is an efficient method for determining mycobacterium tuberculosis.**	The main limitations itslow sensitivity, inabilityto allow species identification and drug susceptibility testing.

3.)	**Interferon-gamma Release Assays (IGRAs)** **a.) Quanti- Feron TB** **b.) T spot TB/ELISPOT assay**	• The assay is based on the immune response of the patient to Mycobacterium tuberculosis. • Gold is a quantitative assay to estimate the production of IFN-γ in a blood sample when incubated with Mycobacterial antigens. • This test estimates Mycobacterium tuberculosis by increasing the level of cytokine-producing T cells in the body.	Stimulation of lymphocytes with Mtb antigens occurs in the tube, rather than in the patient's arm. This method cannot differentiate between latent TB infection and active TB disease.

1.3.2 ADVANCED METHODS OF DIAGNOSIS:

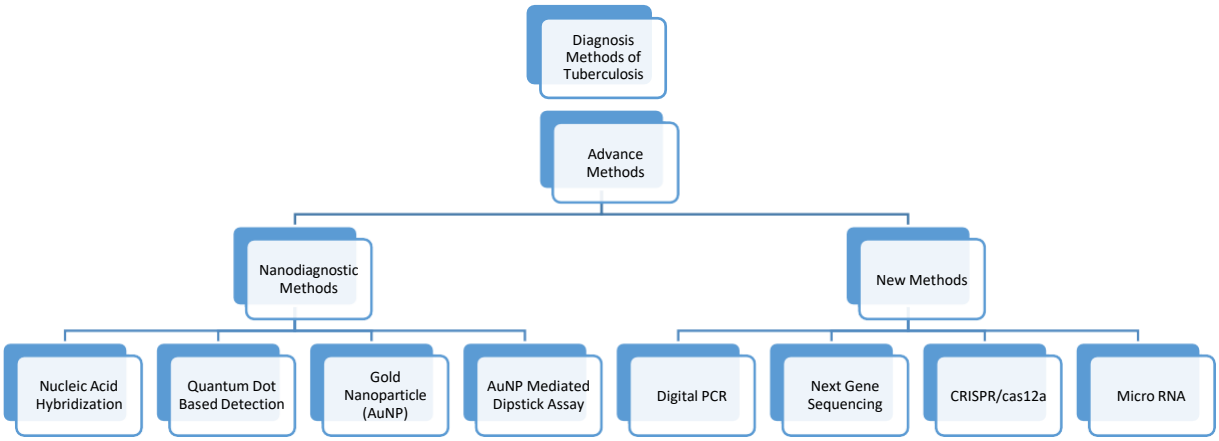


Fig 10:- Flowchart of advanced diagnostic methods for detecting tuberculosis.

Digital PCR:- The third generation PCR method for precisely estimating the amount of nucleic acid in a sample is called droplet polymerized chain reaction. The sample is gathered and divided among several chambers, some of which may have more than one target while the remaining chambers may not have any targets at all. Thermal amplification, in which the goals receive an increase in multiple copies, comes next. The next step is fractionating the sample to produce droplets using fluorescent probes like Eva Green (44), HEX (Hexachloro-Fluorescein), and FAM (Fluorescein amidite). Following the production of roughly 20,000 droplets, it is suspended in an emulsion of water and oil. While some droplets may contain no target genes at all, others may contain multiple target genes. An endpoint thermal cycler is used to amplify the sample droplets. The droplets are put in an analyser after amplification in order to identify target genes based on the color that the fluorescent dyes produce. These dyes produce signals, which are measured and categorized into positive and negative fractions. The desired gene of interest is present in the positive fraction, while it is absent in the negative fraction. Typically, the process of reading is carried out out in a Quanta soft software analyser. After completing the droplet reading, data analysis is carried out. It is possible to analyse, visualize, and export the data .

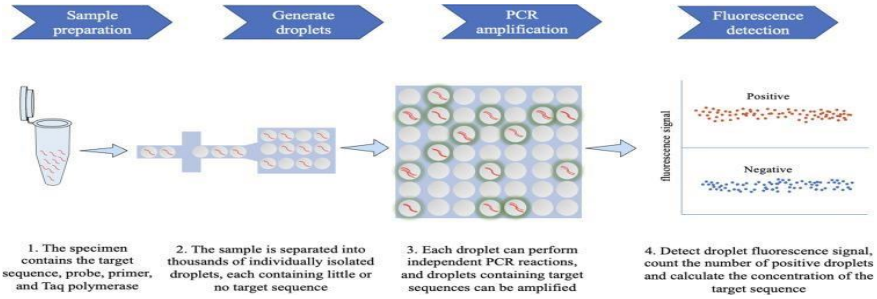


Fig 11:- Showing process of digital PCR for detection of tuberculosis.

Next gene sequencing:- Metagenomic sequencing, or next gene sequencing (NGS), is a sophisticated method that produces billions of DNA molecules and can be used to identify antibiotic resistance in tuberculosis infections. This method can be used to increase the expression of drug-resistant genes. When it comes to identifying mutations linked to resistance to isoniazid, rifampicin, streptomycin, ofloxacin, levofloxacin, and moxifloxacin, this technique is extremely sensitive. The entire procedure can be divided into multiple phases, including sample preparation, DNA library construction, sequencing, and data interpretation. First, a patient sample is taken, and the nucleic acid is extracted using a nucleic acid extraction kit. NGS carries out the sequencing using a flow cell that contains a number of tiny microbeads. To ligate the extracted nucleic acid, an adaptor sequence related to many oligonucleotides on the microbeads is used. The oligonucleotides in the flow cell will bind to the adaptor sequence. The reaction mixture is enriched with DNA polymerase to amplify the extracted DNA primers, or dNTPs. Therefore, amplification results in the formation of double-stranded DNA. Denaturation and circularization are the processes that turn the expanded DNA sequence into a ssDNA. Using rolling circle amplification, one can produce DNA nanoballs for readings (long or short) and single nucleotide identification.

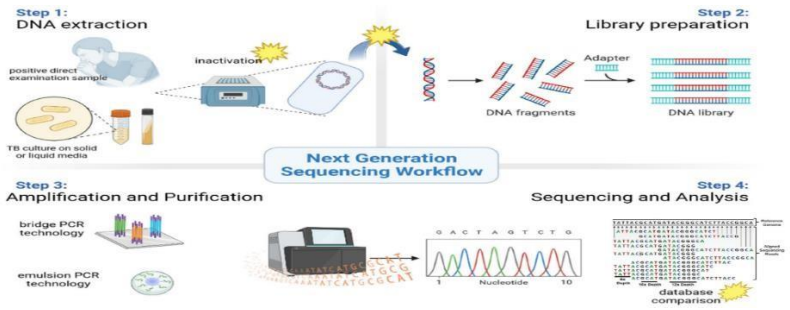


Fig:- 12 Showing process of Next gene sequencing for detection of tuberculosis

CRISPR/cas12a:- The CRISPR Cas system gained popularity due to the pressing need to quickly develop an appropriate diagnostic technique that could identify Mtb in patient samples. A palindromic DNA sequence that is short and repeats itself, CRISPR is responsible for encoding the Cas proteins. The element of the bacterial adaptive immune system is CRISPR Cas. Only recently has the use of CRISPR in Mtb diagnosis been implemented. Nucleotide activators, an enzyme domain, and a guide RNA probe are shared by various Cas protein variants. For instance, the Cas12 protein frequently cleaves single-stranded DNA because it views double-stranded DNA as an activator. While Cas 14 cleaves act on ss-DNA, Cas 13 recognizes ss-RNA . However, the adjacent motif of the protospacer (PAM) is the 2–6 bp nucleotide sequence that gives the Cas proteins their specificity. As a result, they are able to identify even very small amounts of nucleic acid in patient samples. Xu et al. proposed a highly sensitive fluorescence-based detection method. Recombinase polymerase amplification (RPA) and CRISPR Cas 12a were combined in this method to identify Mtb DNA with specificity and sensitivity. In comparison to the traditional culture method, the assay demonstrated excellent specificity (100%) and sensitivity (99.29%). CRISPR demonstrated exceptional diagnostic efficacy across a range of specimens obtained from cerebrospinal fluid and sputum. CRISPR Cas has shown to be an extremely sensitive and promising treatment for extrapulmonary and pulmonary tuberculosis. First off, this technique's complexity is incredibly low, making it compatible with the existing methods for boosting effectiveness . Owing to its extreme sensitivity, CRISPR is able to identify several drug response sites at once, primarily isoniazid and rifampicin. Additionally, it can offer genetic information about drug resistance to both first- and second-line medications.

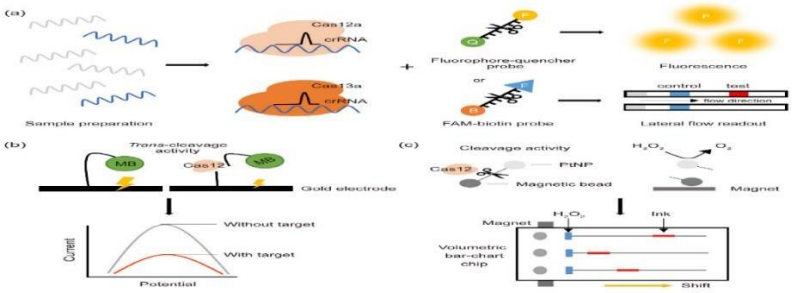


Fig:-13 Process of CRISPR/cas 12a

MicroRNAs:-

MicroRNAs are very conserved single-stranded, non-coding RNA molecules that range in length from 18 to 25 nucleotides. They are necessary for cellular differentiation as well as the start of both innate and adaptive immunity. can thus be utilized as a biomarker in medicine. Numerous diseases, including tuberculosis, are treated, diagnosed, and prognosed using microRNAs as biomarkers. They take part in a number of biological processes. The variations in circulating mRNAs among individuals are negligible, constant, and insensitive to endogenous RNAse activity. studies on the expression of microRNA in plasma samples from pulmonary tuberculosis patients. This plasma-based detection technology offers a practical way to diagnose tuberculosis in a population where getting sputum might be difficult, especially in cases of extrapulmonary TB and pediatric patients .

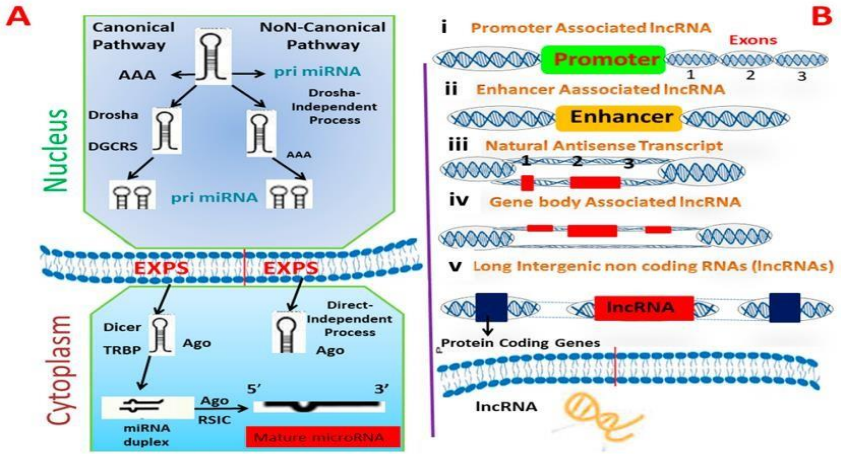


Fig 14:- Image of micro RNA used for the diagnosis of tuberculosis.

NANOMATERIAL FOR TUBERCULOSIS DIAGNOSIS:-

Diagnostic techniques based on nanotechnology would offer quick and effective point-of-care testing, resulting in successful TB treatment and eradication. Because of the special qualities of nanoparticles for the identification of TB biomarkers, there has been a notable advancement in TB nanodiagnostics during the past ten years .

Nanoparticles are extremely small particles with a diameter of less than 100 nm that possess special qualities not found in other atoms or materials. Among the most significant characteristics are a large surface-to-volume ratio (gold nanoparticle), strong

Surface catalysis, high electrical and thermal conductivity, improved photoluminescence, and Surface-improved Raman Scattering (SERS) .

Nucleic acid hybridization nano-diagnosis: The technique for detecting mycobacterial DNA using nucleic acid hybridization uses both modified and unmodified nanoprobes.

Gold nanoparticles can be used in unmodified nanoprobe-based detection to find Mtb by utilizing particular nanoprobes that can isolate the bacteria, like Au, Ag, and Zr. For instance, particular oligonucleotides complementary to the desired gene can be designed in order to detect 16s rRNA. Because it can identify unamplified DNA from clinical samples, it is very beneficial. They are also the best option for diagnosis because of their sensitivity . Conversely, the modified method makes use of immobilized DNA probes tailored to the target gene on nanostructured metal oxides, like zirconia (ZrO2). Zinc Oxide (ZrO2) is an inorganic metal oxide that possesses several remarkable properties, including great thermal stability, chemical inertness, non-toxicity, and remarkable affinity for oxygen-containing groups .

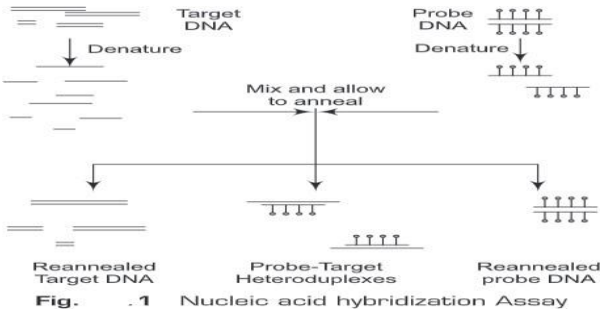


Fig 15:- Depicting process of Nucleic acid hybridization.

Quantum dot based detection:-The development of diagnostic platforms frequently makes use of quantum dots, also known as semiconductor-based nanocrystals, because of their slow decay rates, narrow emission spectra, and broad absorption spectra. In a clinical setting, they have the rare capacity to simultaneously obtain several targets in a patient's sample. They are generally favored over standard fluorescence-based methods. Sulfurous acid, chromium quantum dots, and a molecular probe particular to the IS900 conserved sequence in Mtb are examples of optional treatments . The molecular probes' combination with the specific target sequences forms a sandwich-like structure that, when exposed to UV light, generates red fluorescence that is visible to the unaidedeye. For improved detecting capabilities, these hybrid detection systems are easily upgraded and highly flexible. Additionally, unamplified DNA from suspected Mtb infection patients can be detected by them. These days, species-specific molecular

probes and CdSeO3 streptavidin are coupled with quantum dots. The sandwich hybridization method (biotinylated probes) is then employed to identify a particular DNA, making it possible to identify Mtb surface antigens. Nevertheless, only 104 cells/ml of the sample are the detection limit .

AuNPS, or gold nanoparticles: Because of their special optical and physicochemical properties (which make them non-toxic and inert), gold nanoparticles (AuNPs) are the ideal nanomaterial for use in multidisciplinary research as well as clinical diagnosis and treatment. When combined with an antibody, antigen, or other biomolecule, the optical property of AuNP renders it ineffective for use in pathogen diagnosis. Furthermore, AuNP does not impair functional activity even in the event of immobilization. Gold nanoparticle surface functionalization improves the antigen-antibody response, boosting immunoassay signals and, ultimately, test sensitivity . It offers a cheap and easy assay that makes it possible to evaluate several samples at once. The assay, which uses AuNP probes (thiol-linked single stranded DNA, or ssDNA, modified gold nanoparticles) to provide colorimetric identification of the target or gene sequence fromtest DNA sample, has been found to be a reasonable alternative to conventional methods of detection, even with very small amounts of mycobacterial DNA. DNA probes, which are oligonucleotides produced from the gene sequence of M. tuberculosisRNA polymerase subunit, were employed in conjunction with AuNP in the first report of the use of AuNP in tuberculosis diagnostics to diagnose M. tuberculosis colorimetrically. Specifically, at 526 nm, the presence of complementary DNA causes the nanoprobe solution to remain pink (no DNA probe aggregation), whereas the absence of complementary DNA in the samples causes the solution to turn purple (nanoprobe aggregation at a high Nacl concentration). The test can be easily be visualized for greater sensitivity than smear microscopy. This method's main benefit is its extremely low chance of contamination extremely precise and produces reliable results.

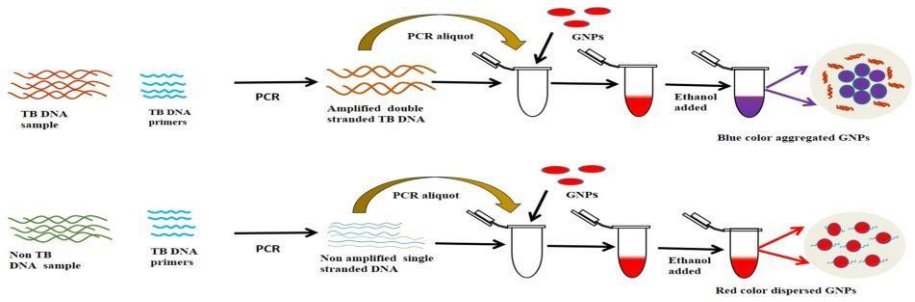


Fig 16:- The process of Gold nanoparticle for detecting tuberculosis.

Biosensor based nano diagnosis:- Biosensors are analytical devices designed to quantify thermal or optical image signals for further investigation. These devices can be used to infer relationships between isolated enzymes, whole cells, tissues, antibodies, and receptor-enzymes. Optical biosensors discover mycobacterial DNA in patient samples using surface plasmon resonance. Surface plasmon-based diagnostics can detect mycobacterial specific antigens (CFP 10) isolated from tissue samples by attaching to immobilized gold nanoparticles on the optical sensor surface. Using this method, anti-CFP10 is limited to the surface of an immunosensor that is cheaply combined with a surface plasmon resonance-based optical immunosensor device. The relationship between the SPR and CFP10 concentration, which is often used as a used as a TB marker, a linear relationship is found. This method is comparatively beneficial, requiring little sample, being highly reusable, and not requiring any pre-treatment .

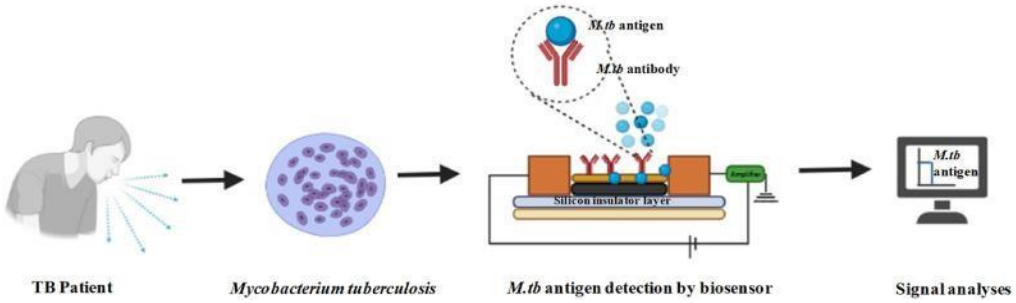


Fig 17:- Detection of tuberculosis by using biosensors.

1.4 IMPACT OF EARLY DIAGNOSIS AND MONITORING OF TUBERCULOSIS PROGRESSION:-

Timely diagnosis and close observation of the advancement of tuberculosis (TB) are essential for enhancing patient outcomes and managing the disease's spread. Approximately 25% of people worldwide are infected with the infectious pathogen Mycobacterium tuberculosis (M.tb). Of those infected, only a small percentage (about 5–15%) go on to develop active tuberculosis (TB), with the majority continuing to harbour a viable latent infection. Genetic studies have identified host factors that drive early progression to active TB compared to those who remain latently infected. Early progression to active TB is a highly heritable trait. Improved early diagnosis may result from the creation of machine learning-based diagnostic algorithms that can differentiate between latent and active tuberculosis infections. Furthermore, a four-protein biomarker panel that can help with early detection has demonstrated 67.3% sensitivity and 96.3% specificity at differentiating between latent and active TB .

Rapid treatment initiation and early diagnosis continue to be essential tactics for tuberculosis control. Both tuberculosis and its spread can be cured and stopped with early diagnosis and treatment. Global goals to lower the number of TB-related deaths and new infections continue to be the main way to measure a country's success in combating the epidemic.

However, in countries with limited resources, diagnosis is hampered by inadequate facilities and unreliable diagnostic methods. Globally, the prevalence of tuberculosis is gradually decreasing; however, if initiatives concentrated on delivering prompt diagnosis and treatment, we could accelerate the process of controlling tuberculosis and lessening its effects .

Impact of Early Diagnosis and Monitoring:-

- **Reduction in Transmission:** The illness spreads through the air when an infected person coughs, speaks, or sneezes. Early diagnosis shortens the time it takes for someone to infect others with tuberculosis, which is especially important in high-density areas like cities, workplaces, and educational institutions. According to research from Peru, early diagnosis and treatment initiation can reduce transmission rates by over 50%, highlighting the critical impact on public health.
- **Improved Treatment Outcomes:** After an early diagnosis, treatment must begin as soon as possible to prevent tuberculosis from getting worse. When tuberculosis is discovered early, before it spreads or seriously damages the lungs, patients usually have better treatment outcomes. The World Health Organization (WHO) has found that earlier treatment is associated with higher rates of cure and lower mortality. Treatment success rates, for instance, were found to be 85% for patients diagnosed early and 60% for those diagnosed later in a Chinese study .
- **Prevention of Drug Resistance:** Drug-resistant tuberculosis (TB), which includes both multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) TB, is an increasing concern. These forms are more difficult and expensive to treat, and often yield worse results. Early diagnosis guarantees that patients receive the appropriate treatment regimens as soon as possible and reduces the likelihood of inappropriate or insufficient treatment, which can lead

to drug resistance. A systematic review found that early detection and appropriate treatment can reduce the incidence of MDR-TB by as much as 30%.

- **Economic Benefits:** The financial burden of tuberculosis includes both direct costs, such as medical care, and indirect costs, such as lost wages. Early diagnosis can lead to significant cost savings. It allows patients to return to work sooner and saves money by reducing the length of time they are sick and disabled. Early intervention also reduces costs by preventing severe diseases that would require more costly, intensive care. In India, early detection and treatment of tuberculosis prevented the nation's healthcare system from suffering annual losses of nearly $1 billion.
- **Reduced Mortality:** Early diagnosis and treatment greatly reduce the risk of dying from tuberculosis-related diseases. This is particularly important in low-resource settings where people might not have easy access to healthcare.
- **Enhanced TB Surveillance:** Early diagnosis and case monitoring of tuberculosis (TB) enable more effective contact tracing and proactive treatment of contacts .

1.4.1.1 Importance of Monitoring:-

- **Treatment Adherence:-** The lengthy nature of TB treatment—which frequently lasts six months or longer—can cause problems with patient compliance. Patients are kept under constant observation to make sure they follow their treatment plans. When healthcare professionals watch patients take their medications under the WHO's Directly Observed Treatment, Short-course (DOTS) strategy, adherence rates rise to over 90%, compared to 60–70% when no such supervision is provided.

- **Detection of Adverse Drug Reactions:-** Mild to severe side effects are possible with TB medications. Frequent monitoring enables medical professionals to identify and address these side effects early on, preserving patient compliance and enhancing the efficacy of treatment. For instance, hepatotoxicity is a typical adverse reaction to medications such as rifampicin and isoniazid. Regular testing for liver function can identify problems early and stop major consequences. Treatment completion rates were 15% higher in the US when side effects were proactively monitored and managed .

- **Assessment of Treatment Efficacy:-** Regular sputum tests, chest X-rays, and clinical assessments are some of the ways that monitoring assists healthcare providers in assessing how well the treatment is working. This guarantees that the TB bacteria is being effectively combated by the treatment. Modifications can be implemented quickly if a patient is not getting better. The ability to monitor and customize treatment has been greatly improved, leading to higher cure rates, with the use of molecular diagnostic tools such as GeneXpert.

- **Psychosocial Support:-** The stigma associated with tuberculosis can result in mental health problems and social isolation. Psychosocial support is crucial and is provided through ongoing monitoring interactions with healthcare providers. Frequent contacts can lessen feelings of loneliness, promote treatment adherence, and offer emotional support. TB patients in Ethiopia who received consistent psychosocial support had a treatment success rate that was 20% higher than that of those who did not.

- **Public Health Surveillance:-** Monitoring provides important data to public health surveillance systems that aid in planning resource allocation, TB epidemiology analysis, and control measure evaluation. Organizations like the WHO rely on accurate surveillance data to support their global tuberculosis control initiatives. Such information is used by the Global TB Report to monitor progress toward the goal of eliminating tuberculosis and to pinpoint regions in need of additional funding and assistance.

CHAPTER 2: LITERATURE REVIEW:-

- **Improving the management of tuberculosis: the purpose of customized, preventative, and predictive medicine** Dohál Matúš et al. * Ivan Solovič et al., Juraj Mokrý et al., and Igor Porvazník et al. published on October 4, 2023, online. The current study suggests multiple specifics therapeutic uses of various devices, such as whole genome sequencing, genetic screening, and artificial intelligence, that significantly alter decisions regarding the clinical management of tuberculosis patients.
- **Current and Traditional Methods for Diagnosing Active Tuberculosis in the Liver** * Novel N. Chegou et al., Anna Ritah Namuganga et al., and Harriet Mayanj Kizza et al. Posted online on September 23, 2021.This study examines the currently accepted testing techniques for pulmonary tuberculosis as well as current research efforts to enhance diagnosis and increase diagnostic technology.
- **The invention of nanodiagnostic technologies from molecules in tuberculosis diagnosis.** Anjali Negi et al., Rashmi Sharma et al., Summaya Perveen et al., and Srestha Mukherjee et al. published online on April 5, 2023. This review aims to investigate the connections between conventional and modern methods to improve sensitivity and specificity even more.
- **Better Standard and Novel Techniques for Tuberculosis Diagnosis** Chuan Wang et al., Jumei Zeng et al., Baoyu Dong et al., Zhiqun He1Yuqing Li et al., Xinyue Xu et al., Publish date: May 31, 2022. The investigation covers the development, assessment, and application of conventional TB testing techniques in addition to the rapid production of novel methods for M. tuberculosis detection.
- **Tuberculosis: Pathogenesis, Current Treatment Regimens and New Drug Targets** Shahinda S. R. Alsayed et. al, and Hendra Gunosewoyo et. al,. Published online 2023 Mar 8. The underlying causes of Mycobacterium tuberculosis, current treatment regimens, and obstacles to TB control methods were assessed in this review article.
- **A novel CRISPR/Cas system-based method for Mycobacterium TB recognizing something.** Zhaobao Pan et al., Xiaoyu Zhang et al., Xiaoying He et al., Yubo Zhang et al., Lei Chen et al., Yueying Huang et al., and Heng Li et al. Printed on October 11, 2023. We developed a specific detection method for MTB in this study using the Clustered Regularly Interspersed Short Palindromic

Repeats (CRISPR) system in combination with recombinase mediated isothermal nucleic acid amplification (RAA) to increase the sensitivity of the detection system and achieve "two-level" amplification of the detection signal.

- **Diagnosis of Tuberculosis: Advances and challenges** Kavitha Sunil Shettigar et al. and Prakruthi Shivakumar et al. examined on August 17, 2022 Printed on November 19, 2022. In this article, we examine the uses and limitations of the currently available traditional and cutting-edge testing methods for tuberculosis screening and diagnosis.
- **The definition of tuberculosis infection according to the range of the illness Altogether** Giovanni Battista Migliori, Catherine W.M. On, Linda Petrone, Lia D'Ambrosio, Rosella Centis, Delia Goletti, and others. 2021 saw publishing. This review covers the many stages, encompassing uninfected people, TB infection, incipient TB, subclinical TB without symptoms, subclinical TB with unnoticed symptoms, and TB diseases with symptoms.
- **A clinical review evaluating the early stages and progression of infection between incipient and subclinical tuberculosis** In 2018, Shuyi Ma et al., Erin Meermeier et al., David M. Lewinsohn et al., David R. Sherman et al., Paul K. Drain et al., Kristina L. Bajema et al., David Dowdy et al., Keertan Dheda et al., Kogieleum Naidoo et al., Samuel G. Schumacher et al. This article examines the immunology, etiology, clinical epidemiology, diagnosis, treatment, and prevention of incipient and subclinical tuberculosis (TB), two freshly identified clinical states of an old bacteria.
- **Cutting-edge laboratory techniques for better drug susceptibility testing and tuberculosis diagnosis** Mugenyi Nathan et al. Joseph Baruch Baluku et al., and Nelson Ssewante et al. Pauline Byakika-Kibwika et al., Alfred Andama et al., Felix Bongomin et al., and Mutuku Mukenya Irene et al. The publication date of this article is January 5, 2024. We have included new laboratory methods for DST and TB diagnosis that are now accessible in this study.
- **A CRISPR–Cas12a-based multiplex detection method for Mycobacterium tuberculosis complex** has been developed and is being evaluated in its first stages by Jing Xiao et al., Jieqiong Li et al., Shuting Quan et al., Yacui Wang et al., Guanglu Jiang et al., and Yi Wang et al. August 25, 2023, saw the publication of this story. For the multiplex detection of MTBC, we invented an easy-to-use, quick, highly sensitive, specific, and economical assay in this work.

When compared to the commonly used Xpert assay, this review paper showed better diagnostic performance.

- **Advanced tailored tuberculosis diagnosis strategies: current clinical methods as well as technology** Tony Y Hu et al., Xuerong Chen et al. January 18, 2021, saw the publication of this article. This review will discuss new methods to personalized tuberculosis diagnosis as well as novel technology from bench to bed.
- **Investigations into diagnostic procedures for infection with TB** Federica Repele et al., Delia Goletti et al., Tonino Alonzi et al. Posted online on August 10, 2023 An update on current methods for creating trustworthy tests to identify TBI, or traumatic brain injury, as well as conditions that proceed from infection to disease is given in this study.
- **A CRISPR-Cas12a-based platform for the clinical detection of Mycobacterium tuberculosis that is ultrasensitive, fast, and highly specific** Xiaming Liu et al., Xiaolan Huang et al., Fei Xiao1 Jin Fu et al., Nan Jia et al., Chaohong Wang et al., Chunrong Sun et al. Posted on May 23, 2023. In order to create the MTB-MCDA-CRISPR, a novel detection platform for tuberculosis diagnosis, we coupled the multiple cross displacement amplification (MCDA) technique with a CRISPR-Cas12a-based biosensing system in this work.
- **Tuberculosis Diagnosis: Nanodiagnostics Methods** S. M. Paul Khurana et al. and Shailendra K. Saxena et al. published on September 25, 2019, online. The present situation of TB diagnostic testing is covered in this chapter, along with the latest developments in nanotechnology-based M. tuberculosis method of detection.
- **A Look at Improvements in Drug Delivery, Vaccines, and Therapeutics Based on Nanotechnology: Multifaceted Uses in the Management of Tuberculosis Disease** Pradipta Ranjan Rauta, Yugal Kishore Mohanta, and Hitesh Chopra, et al. published online on April 12, 2023. In order to effectively eradicate tuberculosis, this article explores the potential applications of nanotechnology in the areas of TB diagnosis, nanotechnology-based pharmaceutical delivery systems, and prevention. It also discusses the drawbacks of utilizing traditional diagnostic techniques.
- **A SHORT REVIEW OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)** October 2023; Nikunj Brahmbhatt Vivek et al.;

Narayan Upasani et al. The present article outlines the basic concepts and techniques of loop mediated isothermal amplification (LAMP), as well as both the advantages and disadvantages of the technique.

- • A narrative review of the use of droplet digital PCR for the detection of mycobacterium leprae and tuberculosis infections Yu Zhang et al., Peng Yue et al., Wenjing Cao et al., Zhenhua Ji et al., Xuan Su et al., Shiyuan Wen et al., Jing Kong et al., Guozhong Zhou et al., Bingxue Li et al., Yan Dong et al., Aihua Liu et al., and Fukai Bao et al. published on March 15, 2022, online. This article discusses the use of ddPCR, a very powerful nucleic acid quantification methodology that does not require a calibration curve, in the diagnosis of tuberculosis, for the detection of treatment resistance, and the tracking of transmission.
- **The Application of Next-Generation Sequencing (NGS) in the Treatment of Tuberculosis: A Feasible Assessment for Continuous. Use** Beviere Marion et al. François Guerin et al., Vincent Cattoir et al., Caroline Piau et al., Malo Penven et al., Loren Dejoies et al., and Sophie Reissier et al. published on July 26, 2023, online. The objective of this study is to provide an accurate, exhaustive, and comparative analysis of the methods available for combining this approach into regular laboratory workflow.
- **Novel Therapeutic Techniques for the Treatment and Management of Tuberculosis Based on Nanotechnology Authors:** Hussein MZ et al., Pratiwi AR et al., Ruman U et al., Kia P et al. Printed on March 8, 2023. The research covers the range of nanotherapeutic compounds that have been produced for vaccines, anti-TB pharmaceuticals, and MTB diagnostics. The idea of using nanoparticles (NPs) in this therapy holds a lot of promise and provides amazing outcomes to defeat the illness.
- **WHO tuberculosis operational handbook. Preventive treatment for TB will be addressed in Module 1**. World Health Organization, Geneva; 2020 publication. Information regarding the positive effects of early diagnosis and progression monitoring of tuberculosis is included in this study.
- **Improvements in functional nanobiosensors for tuberculosis diagnosis: A new approach to tuberculosis control** Xuran Yang and colleagues, Shuhao Fan and associates, Yuhe Ma and associates, Hui Chen and associates, Jun-Fa Xu and associates, Jiang Pi and associates, Wandang Wang and staff members, Guanghui

Chen. et al. Printed on December 15, 2022. The various types of diagnostic targets for tuberculosis diagnosis based on nanobiosensors were covered in this article, encompassing particular cytokines and Kevin Schwartzman et al. Posted online on March 25, 2022. This article assesses the pathology and transmission of tuberculosis, as well as the several factors that determine the occurrence of transmission.

- **Thoraci ultrasound has a complementary role in the diagnosis and therapy of pulmonary tuberculosis through chest imaging.** Gaetano Rea and associates Giovanni Sperandeo et al., Ernesto Giuffreda et al., Maria Pia Foschino Barbaro et al., Roberta Lieto et al., Carla Maria Irene Quarato et al., Beatrice Feragalli et al., Tullio Valente et al., Giulia Scioscia et al., Ernesto Giuffreda et al., and Donato Lacedonia et al. published on December 10, 2021, online. This piece assessed TB Mycobacteria are mostly transmitted by respiratory droplets, with the pulmonary parenchyma typically acting as the first site of infection. Chest imaging consequently becomes crucial for the medical procedure. In locations with limited resources, thoracic tomography (TUS) serves as an inexpensive, radiation-free, portable, and non-invasive tool that is easy to obtain.
- **Quantum Dots in the Diagnostics of Biological and Non-Microbial Disorders: Present Situation and Prospects for the Future.** PR Kumar et al. Pooja Kumar Ramesh et al.Date of The publication: April 4, 2023. The most modern and promising quantum dot technologies have been addressed in this article, such as QD-based biomarkers, QD-based biosensor devices, QD-based paper strip devices, and QD-based lateral flow immunoassay. In present biomedical applications, quantum dot-based devices and practices are appealing add-ons. Such techniques will pave the way for next-generation diagnostics.

CHAPTER 3: METHODOLOGY:

RESEARCH GAP:-

1.) This study is conducted to know the impact of early diagnosis and monitoring on tuberculosis progression.

2.) The study is conducted to know different diagnosis methods for detecting tuberculosis.

3.) The study is conducted to know the new methods used for diagnosis of tuberculosis infection.

4.) To know what are the limitations of conventional methods of diagnosis for detectingtuberculosis?

RESEARCH QUESTION:-

1.) What is the impact of early diagnosis and monitoring of tuberculosis progression?

2.) What are the conventional methods used in the diagnosis of tuberculosis and what are the limitations of using conventional methods?

3.) What are the advanced and nano-diagnostic methods used in the diagnosis of tuberculosis?

4.) What is the importance of monitoring tuberculosis?

5.) What are the different stages of tuberculosis infection and its progression from latent TB to active TB?

RESEARCH OBJECTIVE:-

1.) The objective is to study the impact of early diagnosis and monitoring of tuberculosis progression.

2.) To study different conventional methods used for diagnosing tuberculosis and what are the limitations of these methods.

3.) To study the advanced and nao diagnostic methods used in the diagnosis of tuberculosis.

4.) To study the importance of monitoring tuberculosis.

5.) To study different stages of tuberculosis and its progression from latent TB to active TB.

RESEARCH TYPE:-

The sort of analysis used for this project comprises of an initial exploratory research followed by a descriptive study. This exploratory study assesses the present methods for diagnosing and monitoring tuberculosis (TB), highlights the limitations of conventional methods, and looks into the potential of new tools. This helps provide a comprehensive understanding of the problem and its solutions. For the descriptive study, information is collected and analysed regarding the actual impacts of early diagnosis and monitoring on tuberculosis outcomes, such as treatment success, disease spread, and financial burden. Combining exploratory and descriptive studies provides a solid foundation for well-informed recommendations.

CHAPTER 4: RESULT AND DISCUSSION

The result of the study conduct shows that advanced diagnostic methods are very helpful in determining tuberculosis as they are not time consuming which results in the proper and timely diagnosis of the infection. The conventional methods used takes time and have low specificity as compared to advanced methods. Moreover, the early diagnosis and monitoring of TB progression are crucial for improving patient outcomes, reducing disease transmission, enhancing surveillance and contact tracing, decreasing economic burden, and advancing diagnostic tools. These strategies are essential for achieving global TB control and elimination goals, particularly in high-burden countries. We used different methods for detecting tuberculosis such as smar microscopy, loop mediated isothermal amplification, tuberculin skin test etc. which are used for diagnosis purpose. There are advanced methods which are being used now and shows result within 24 hr of tests. This will eventually help in proper diagnosis of TB infection and will also reduce the chances of death from tuberculosis. The findings of this study show how important it is to diagnose and monitor tuberculosis (TB) early. TB is a big problem around the world, with millions of people getting sick and many dying from it each year, but the findings suggest that catching TB early can make a big difference. One of the key points is that when TB is diagnosed and treated quickly, patients have a much better chance of getting better. They are less likely to have complications or die from the disease. This shows how crucial it is to improve our ability to detect TB early and make sure everyone gets the treatment they need. By providing the right treatment at the right time, we can not only help people recover but also stop TB from spreading to others. One of the key points is that when TB is diagnosed and treated quickly, patients have a much better chance of getting better. They are less likely to have complications or die from the disease. This shows how crucial it is to improve our ability to detect TB early and make sure everyone gets the treatment they need. By providing the right treatment at the right time, we can not only help people recover but also stop TB from spreading to others. One of the strengths of the study is that it looks at the progress being made in TB diagnostic tools, such as the loop mediated isothermal amplification test. These new technologies are faster, more accurate, and more widely available than traditional methods. They can help bridge the gaps in detecting TB early and monitoring its progress. However, the study also acknowledges that there are challenges in using these strategies, such as limited access to diagnostic tools, lack of training, and high costs.

LIMITATIONS OF CONVENTIONAL METHODS USED FOR TUBERCULOSIS DIAGNOSIS:-

Because drug-resistant tuberculosis (DR-TB) shares many clinical and pathological characteristics with drug-susceptible tuberculosis, it is imperative that DR-TB be confirmed through microbiological and/or molecular methods. Drug-resistant tuberculosis is no more contagious and spreads similarly to drug-susceptible tuberculosis. However, prolonged infectious periods or a delay in diagnosing drug resistance may facilitate further transmission and the emergence of antibiotic resistance [40].

It is possible to perform routine drug susceptibility testing (DST) and culture using phenotypic or genotypic approaches. Phenotypic testing on mycobacteria that are in the active growth phase in the culture media is required, so the results won't be available for at least two to three weeks if liquid media are used, and up to four to eight weeks if solid media are used. In addition to having higher contamination rates, solid media call for a large amount of supplies, media, and skilled labor. This wait may not be warranted if a decision needs to be made regarding the patient's optimal course of treatment. On the other hand, molecular testing yields results by using genetic amplification techniques to identify mutations in genes encoding resistance to anti-TB drugs in 24–48 h. As a result, whenever these tests are available, all TB patients should have them performed. In terms of obtaining results, DST's molecular procedures are significantly faster than culture-based methods .

The traditional techniques for diagnosing tuberculosis (TB), such as sputum microscopy, drug susceptibility testing, culture testing, and radiology, have demonstrated the ability to identify acid-fast bacilli, offer point-of-care diagnostics, and are reasonably priced. The main drawbacks of these diagnostic techniques, however, are their low sensitivity, lengthy processing times, false negative results, low efficacy, inability to distinguish between various strains, and inability to detect bacterial viability.

This causes a delay in the diagnosis of tuberculosis. Postponed diagnosis exacerbates the infection, raises the death risk, and allows bacilli to spread throughout the healthy population.

Furthermore, an inaccurate diagnosis will result in ineffective treatment, which will ultimately cause the afflicted person to become drug-resistant. Therefore, it is necessary

to investigate quick, extremely sensitive, and targeted diagnostic techniques and methods for Mtb detection .

The most common technique for diagnosing tuberculosis, TST, has several significant disadvantages. Lack of specificity with TST is the main cause for concern. Due to the test's inability to distinguish between Mtb infection and non-tuberculosis mycobacteria or vaccination with Bacille Calmett-Guérin (BCG), false-positive results are frequently obtained.

Cross reactivity, in which an immune response is triggered by homologous antigens from either the BCG vaccination or environmental non-pathogenic mycobacteria, is typically to blame for both instances of false positive responses. TST is still the most widely used method for identifying Mtb infection in spite of these issues. Within Quanti FERON-TB Rather than in the patient's arm, lymphocytes are stimulated with Mtb antigens in the phlebotomy tube. Therefore, unlike in the case of the tuberculin skin test, there is no booster effect from repeated testing.

CHAPTER 6: CONCLUSION

With the help of this review we concluded that it is significant to have proper diagnosis and monitoring of tuberculosis infection which will help to reduce the spread of infection that can be contagious. If tuberculosis is diagnosed and treated quickly, so the chances of patient survival and recovery increases and it also reduces complication. The conventional methods used for the diagnosis are chest radiography, sputum smear microscopy etc. are used earlier for the diagnosis but recently used methods are CRISPR/cas12a, micro RNA, gold nanoparticle etc. which enhances the chances of detecting tuberculosis infection in a limited time as compared to conventional methods.

We also studied the impact of early diagnosis and monitoring which will help in reducing disease transmission, improve treatment outcome, prevention form drug resistance.

The recommendations of the review is that, we can provide personalized treatment to the patients which will help in advancing the treatment for the patient. Personalised treatment will make sure that the patient receives specific medication which will have a bigger impact on patient's health and the recovery will be faster resulting in very low recurrence of the disease.

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