SUITABILITY OF TEST METHOD AMONGST THE VARIOUS TEST METHODS AVAILABLE FOR DETERMINATION OF EXTRAPULMONARY MYCOBACTERIUM TUBERCULOSIS (EPMTB)

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ABSTRACT

Extrapulmonary Mycobacterium tuberculosis (EPMTB) is distinguished by its atypical appearance, varied anatomical involvement, and paucibacillary feature of specimens, all of which contribute to the significant diagnostic challenge it presents. For the purpose of diagnosing EPMTB, it is of the utmost importance to evaluate the suitability and effectiveness of the many diagnostic modalities that are available. These modalities vary from the conventional smear microscopy and culture methods to the most cutting-edge molecular technologies such as GeneXpert MTB/RIF and PCR-based assays. By comparing and contrasting the sensitivity, specificity, turnaround time, accessibility, and cost-effectiveness of various test procedures, the objective of this study is to investigate the diagnostic efficacy of various test techniques in a variety of clinical settings. An assortment of diagnostic procedures were carried out on a collection of extrapulmonary specimens, which comprised tissue biopsies, cerebrospinal fluid, pleural fluid, and lymph node aspirates, among other things. The analysis of the data allowed for the determination of the testing method that was the most appropriate for providing a speedy and accurate diagnosis.

Keywords: Extrapulmonary Tuberculosis (EPMTB), Mycobacterium tuberculosis, Diagnostic Methods, Molecular Testing,

INTRODUCTION

The wide variety of clinical manifestations that can occur with extrapulmonary Mycobacterium tuberculosis (EPMTB) infections, as well as the difficulty in acquiring specimens that are acceptable for testing, these diseases provide substantial diagnostic hurdles. One can choose from a number of different diagnostic approaches, each of which has both advantages and disadvantages. A number of considerations, including sensitivity,

specificity, turnaround time, accessibility, and the makeup of the sample that is being collected, are taken into account while selecting the most appropriate technique of testing. Due to their capacity to directly identify Mycobacterium tuberculosis (MTB), conventional techniques like as microscopy with Ziehl-Neelsen (ZN) staining, culture on Lowenstein-Jensen (LJ) medium, and radiometric BACTEC culture continue to be utilised in a significant manner. These approaches, on the other hand, have a number of limitations, including a poor sensitivity in paucibacillary samples, longer incubation durations, and the demand for biosafety procedures, which makes them less appropriate for quick and early detection.

Overview Of Mycobacterium Tuberculosis (MTBC)

The bacterial pathogen known as Mycobacterium tuberculosis (MTB) is a highly specialised bacterium that is principally responsible for the disease known as tuberculosis (TB), which continues to be a significant public health problem all over the world. These closely related species include Mycobacterium bovis, Mycobacterium africanum, Mycobacterium canettii, and Mycobacterium microti, amongst others. MTB is a member of the Mycobacterium tuberculosis complex (MTBC), which also comprises other species. Because of its distinctive cell wall composition, which is abundant in mycolic acids, the bacterium is an obligate aerobe, slow-growing, and acid-fast. This is one of the factors that contributes to its resistance to desiccation, disinfectants, and immunological reactions of the organism. As a result of infected droplets being discharged into the air whenever an individual with active pulmonary tuberculosis coughs, sneezes, or talks, the most common method of transmission of tuberculosis is through the air. After being breathed, MTB is able to travel to the alveoli of the lungs, where it is phagocytosed by macrophages that are found in the alveoli. However, rather than being eradicated, the bacteria has developed complex processes that enable it to live and proliferate within macrophages. This enables it to circumvent the immune system of the host and form an infection.

Pathophysiology

As a result of the nuclei of inhaled droplets being deposited into the terminal airspaces of the lung, transmission occurs. When the TB count is between 1,000 and 10,000, a TST reaction may identify a cellular immune response due to the presence of the disease. Typically, this occurs between two and twelve weeks after the infection has been present. In order to activate Langerhans cells, lymphocytes, and polymorphonuclear leukocytes, mycobacterium tuberculosis (MTB) utilises a wide range of cell wall components, such as glycoproteins, phopholipids, and wax D. The degree to which they are biocompatible.

Epidemiology

It is still one of the top causes of mortality throughout the world that tuberculosis (TB) contributes to. Twenty-three percent of the world's population has been afflicted with tuberculosis. 3, There are around 9 million people in the United States who are living with a life-threatening brain injury. In 2011, around 1.4 million individuals lost their lives due to TB. Among those who are living with HIV, tuberculosis (TB) is the most common cause of

death. People who were born in a different nation have a chance of developing TB that is 10 times greater than someone who was born in the United States. This is in the United States of America.

Transmission

The transmission of tuberculosis takes place by the inhalation of droplet nuclei. Despite ongoing efforts to reduce the spread of tuberculosis, transmission from person to person continues to pose substantial challenges to public health. In particular, when cavitary disease is evident or when the sputum is positive for AFB smear, individuals who have undetected active laryngeal or pulmonary illness that is not being treated are infectious. Similar to the previous example, people who have sputum smear-negative pulmonary tuberculosis but culture-positive TB may also transmit infection.

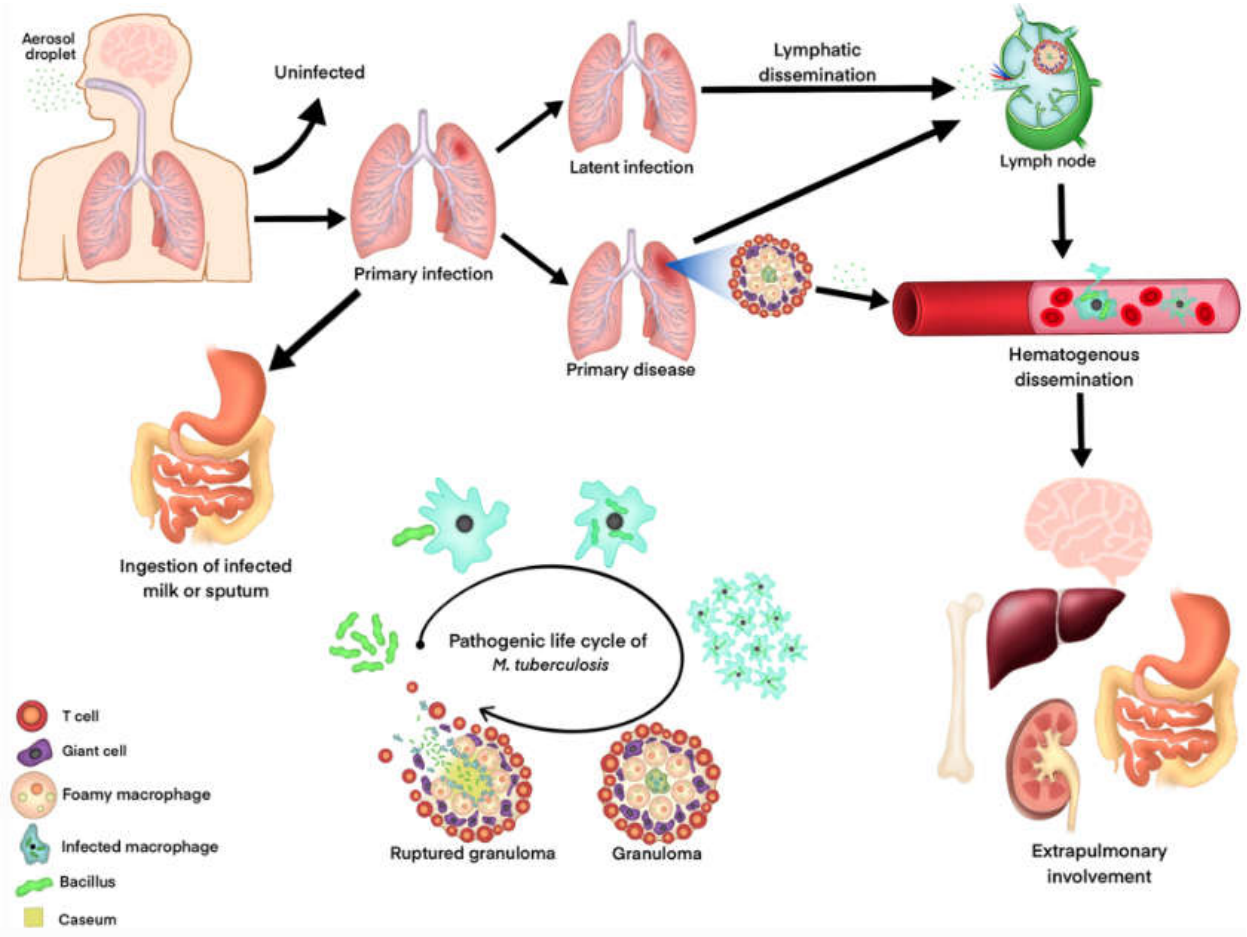

Figure 1: pathogenic life cycle and extra pulmonary dissemination of m. Tuberculosis

Mycobacterium Tuberculosis Complex Evolution

There is a high probability that the Mycobacterium tuberculosis complex (MTBC) originated on the continent of Africa, namely in the Horn of Africa. Mycobacterium tuberculosis, Mycobacterium bovis (Dassie's bacillus), Mycobacterium caprae, Mycobacterium microti, Mycobacterium mungi, Mycobacterium orygis, and Mycobacterium pinnipedii are some of

the other members of the MTBC that are capable of infecting animals. There is a possibility that the M. canettii clade is connected to this group as well. Despite the fact that these animal MTBC strains are closely related to one another and are a member of the M. tuberculosis lineage, they are currently regarded as species owing to historical reasons, even though they do not fit the criteria for species status scientifically.

REVIEW LITERATURE

Namrata (2022), There is an increase in the prevalence of extrapulmonary tuberculosis, which contributes to the global pandemic of tuberculosis. A diagnosis of extrapulmonary tuberculosis is considerably more difficult to make than a diagnosis of pulmonary tuberculosis. The objective of this study is to assess the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of three diagnostic methods that are used for the aim of identifying Mycobacterium tuberculosis in patients who are diagnosed with extrapulmonary tuberculosis (EPTB). These instruments consist of the GeneXpert test, LED-FM, and ZN staining, among others. IGIMS in Patna, which is located in the state of Bihar in India, was the location where this prospective study on microbiology was carried out. ZN-stain, LED-FM, GeneXpert test, and MGIT culture were done on samples that were collected from 210 cases that were deemed to be extrapulmonary.

OBJECTIVE

1. Listing of various conventional and modern art technique used for identification of EPMTB detection.

2. Significance of Detection Technique to understand there utility according to phase of disease.

RESEARCH METHODOLOGY

In this study, we will enrol individuals who are suspected of having thalassaemia based on clinical symptoms, family history, and initial haematological findings. We will specifically target patients who have microcytic hypochromic anaemia, and we will exclude patients who have a confirmed diagnosis of hemoglobinopathies that are not related to thalassaemia. Within twenty-four to forty-eight hours, EDTA tubes will be used to collect three to five millilitres of venous blood samples. These samples will be stored at temperatures ranging from two to eight degrees Celsius. In the beginning, every sample will be put through a complete blood count (CBC) with the assistance of an automated haematology analyser. This will involve the measurement of various parameters, including haemoglobin concentration, mean corpuscular volume, and other red blood cell indices. This will ensure that only samples that meet the quality control criteria will be allowed to proceed with further analysis. After that, High-Performance Liquid Chromatography (HPLC) will be utilised as the primary method for separating and quantifying haemoglobin fractions.

Study Design and Population

The people who are suspected of having Thalassaemia will be the subjects of this research. The clinical symptoms, family history, and preliminary haematological results will all be taken into consideration. Patients who have been referred to haematology clinics or diagnostic centres for additional investigation of anaemia and abnormal haemoglobin levels will be included in the research population. The patients who meet the inclusion criteria will be those who have microcytic hypochromic anaemia, anaemia for which there is no known cause, or a history of hemoglobinopathies in their family. Patients with known iron deficient anaemia who do not have a history of haemoglobin abnormalities will be excluded from the study. Patients who have previously been diagnosed with Thalassaemia major or other hemoglobinopathies will also be excluded.

Sample Collection and Preparation

In order to prevent the blood from clotting, a sample of three to five millilitres of venous blood will be collected from each participant and put in tubes containing ethylenediaminetetraacetic acid (EDTA). A temperature range of 2 to 8 degrees Celsius will be maintained for the samples, and they will be examined within a time frame of 24 to 48 hours. This will ensure that the measurement of the haemoglobin fraction is taken accurately. An automated haematology analyser will be used in order to carry out a complete blood count (CBC) prior to the implementation of the analysis. This will make it possible to evaluate a wide range of factors, such as the levels of haemoglobin (Hb), the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH), and the red blood cell (RBC) indices.

RECSULT

An automated software program will be used in order to do an analysis on the HPLC chromatograms that were obtained. The abnormal haemoglobin peaks will be confirmed by comparing them to the retention periods that are believed to be standard. When doing statistical analysis, either SPSS or GraphPad Prism will be used as the software of choice. In order to analyse the differences between the various patient groups, descriptive statistics, such as the mean and standard deviation, will be utilised for quantitative data. Chi-square or t-tests will be utilised for the purpose of analysing the differences.

Blood samples collection

In order to conduct an examination, blood samples were collected from one hundred patients at the Central Health Public Laboratories. Additionally, blood samples were collected from the patients' family members and relatives who had travelled from Baghdad during this time.

Buffers and solutions : (Betke et al., 1959) An estimation of the HbA2 level

The HbA2 estimate process makes use of the following buffers:

Barbitone buffer (pH 8.9)

Sodium-diethylbarbiturate	5.15 gm
Diethylbarbituric acid	0.92 gm
Distilled water	1000ml

In order to dissolve sodium-diethylbarbiturate and diethylbarbituric acid, a volume of distilled water equal to 500 millilitres was used. After that, the total volume of the solution was increased to one thousand millilitres by adding distilled water, and the pH of the solution was adjusted to 8.9.

Tris (pH 8.9)

Tris(hydroxymethyl)amino methane	14.5gm
Ethylene diamine tetra acetic acid	1.5 gm
Boric acid	0.9 gm
Distilled water	1000ml

One thousand five hundred millilitres of distilled water was used to dissolve three different substances: tris(hydroxymethyl)amino methane, ethylene diamine tetraacetic acid, and boric acid. In order to get the volume of the combination up to 1000 millilitres and bring the pH of the mixture down to 8.9, more distilled water was added as required.

Lysate solution

Obtaining blood samples and placing them in tubes that had been coated with anticoagulant, with Na2EDTA functioning as the anticoagulant, was the procedure that was applied. Following three washes with saline at a concentration of 0.85%, red cells were lysed by adding two volumes of distilled water after each washing. This was done in order to remove any remaining cellular debris.

Normal saline

Sodium chloride	8.5 g
Distilled water	1000ml(pH7)as final volume

An electrophoresis of haemoglobin was carried out by Dacie and Lewis (2001) utilising cellulose acetate paper as the medium.

When employing cellulose acetate paper for haemoglobin electrophoresis, the following buffers and solutions were used in order to accomplish the method.

Barbitone buffer pH 8.6

Sodium diethylbarbiturate	5.15gm
Barbitone (diethylbarbituric acid)	0.92gm
Distilled water	1000ml

Following the dissolution of barbitone and sodium-diethylbarbiturate in 500 millilitres of distilled water, the pH was lowered down to 8.9, and the volume was increased to 1000 millilitres by adding more distilled water.

Hb electrophoresis by agarose gel (HYDRAGEL HEMOGLOBIN (E) K20 KIT)

Lysate solution: The procedure of collecting blood samples in tubes that had been anticoagulated included the use of Na2EDTA, which is an anticoagulant. These samples were then rinsed twice with ten litres of saline during the whole process of collection. After that, the samples were collected. A combination that consisted of one hundred thirty microlitres of Hemolyzing Solution and ten microlitres of packed red cells was mixed in order to achieve the goal of hemolyzing. The mixture was then allowed to rest at room temperature for five minutes after being mixed in a vortex for 10 seconds. After that, it was allowed to settle before being stirred again.

Agarose gel

agarose	0.8 g/100ml
Alkaline buffer	pH 8.5±0.1

Electrophoresis was carried out on gel plates that were prepared for use just before the procedure began.

An aqueous solution that contains tris-barbital

Barbital	2.45%
Sodium barbital	13.73%
Sodium azide	0.13%

Electrophoresis buffer was produced by diluting each fifty millilitres of water with distilled or deionised water until the volume reached one litre. This process was repeated until the buffer reached the desired length.

Working solution for electrophoresis: The electrophoresis buffer and sodium azide are both components of the working solution for the electrophoresis application.

Amidoblack stain

Amidoblack	0.4g/ 100ml
Ethylene -glycol	6.7%

The electrophoresis buffer and sodium azide are both components of the working solution for the electrophoresis application.

Staining solution diluent

Furthermore, this was used in the process of producing the solution for the amidoblack staining.

Destaining solution: Each vial of stock destaining solution has to be diluted with distilled or deionised water to a volume of up to one hundred litres before it can be used. utilising just one millilitre of the solution, the stock solution may be diluted to a volume of one litre. This can be accomplished by utilising the solution. In the aftermath of the dilution process, the destaining solution that the working solution is composed : Citric acid 0.05 g/100ml

Depending on the circumstances, the stock of the destaining solution was stored either at room temperature or in the refrigerator. Should the packaging of the kit or the label on the vial of the destaining solution have an expiration date, the product will continue to be stable until the date specified on the label. It is possible to maintain the stability of the working destaining solution for a period of one week if it is kept in a container that is sealed and kept at room temperature.

Hemolyzing solution: Hemolyzing solution is a buffer that contains additives; it is nonhazardous at the concentration that is used; it is essential for optimal performance; it is typically maintained at room temperature or in the refrigerator; it is stable until the expiration date that is mentioned on the kit packaging or the label of the vial of Hemolyzing solution; and it is essential for optimal performance.

Fixative solution

This solution contains (vol. /vol.):

60% ethanol

10% acetic acid
30% distilled or deionized water

Following the completion of the thorough mixing process, it was stored at room temperature, tightly sealed to prevent evaporation, and disposed of after a period of three months as per the requirements.

MS9 devise (automatic full digital cell counter) (BIO – RAD)

The use of this method allowed for the acquisition of a full blood picture, which included the measurement of haemoglobin, platelet count, platelet differential count, platelet count, platelet count, and platelet count. Electrical impedance technology serves as the basis for the method's functioning, which is based on the concept of electrical impedance technology.

Hb analysis by variant beta thalassemia short program

A sample of blood is put through this procedure in order to get a variety of Hb structures along with the percentages that correspond to each of those structures. With this procedure, Hb separation may be carried out in an automated manner. High-Performance Liquid Chromatography (HPLC) is a technique that takes use of the cation-exchange idea to separate different types of haemoglobin molecules. These Hb molecules include, but are not limited to, HbA, HbA2, HbF, HbS, HbC, and HbD.

Leishman stain (Frei et al., 1995)

Leishman powder	1.5 gm
Methanol (absolute)	1000 ml

DNA extraction from entire blood was performed. The combination was first heated to a temperature of fifty degrees Celsius, then allowed to cool at room temperature, and then shook many times during the course of the day. In order to get a stain that was clear, it was filtered after being allowed to stand for twenty-four hours.

To separate DNA from whole blood, the AB ANALITICA kit was used as the instrument of choice:

Solution 1 (lysis reagent)
Solution 2 (washing buffer 2)

Solution 3 (washing buffer 3)
Filter columns
Tubes and caps

Solution 1 should be shaken before use to resuspend the binding resin.

PCR Amplification

In order to carry out the PCR amplification, the following reagents were selected and used.

The following are starters: This table (1), which was provided by Alphadna Company, contains the primer sequences that have been acquired. In addition to being ready for use, these primers had previously been prepared for their intended use.

Table 1. Primers sequences used in PCR amplification.

P5	CCAACTCCTAAGCCAGTGCC
P7	CTTTCCCTAATCTCTTTCTTTCAGGGC
P10	CACTGACCTCCCACATTCCC
P12	CTGAGACTTCCACACTGATGC

(10x) PCR buffer

This buffer, which was provided by aj ROBOSCREEN Company, is made up of 10 millimolar Tris(HCl) with a pH of 8.3, 50 millimolar potassium chloride, and 0.0001 percent gelatin. This particular combination of dNTPs, which was provided by aj ROBOSCREEN Company, has a concentration of 2.5µmol per sample.

Polymerase of Taq (Paq)

Aj ROBOSCREEN Company provided the enzyme in a concentration of 5 units per microlitre of the sample. This concentration was used in the analysis.

For the purpose of this experiment, the Bsu 36I restriction enzyme that was given by Promega Company was used at a concentration of 10 units per 5 microlitres.

Guidelines for the process of sterilising

Within the context of the autoclaving procedure, buffers and solutions were sterilised by means of a pressure vessel (autoclave) at a temperature of 121 degrees Celsius and 15 ib/in2 for a period of fifteen minutes.

Sterilisation of glassware was achieved by the application of dry heat, which was carried out in a laboratory oven. Before placing the glassware into the oven, the temperature was adjusted to 180 degrees Celsius for a period of two hours.

For the preparation of the Hb electrophoresis, a paper made of cellulose acetate was used, as Dacie and Lewis explained in their 2001 publication.

The process of preparing the blood lysate in compliance with the specification (2.1.4.1.3).

After shaking the precipitate with one's hands for a period of two minutes, distilled water was added to the precipitate in a volume that was twice as big as the precipitate. After that, the combination was left undisturbed for a period of one hour. To begin, five or six drops of chloroform are added to the mixture. After that, the combination is shaken for a period of four minutes. Finally, the mixture is centrifuged at a speed of 3500 revolutions per minute for a period of fifteen minutes. At the time of the lysate extraction, the Pasteur pipette was used. During the time when the power supply was still disconnected, the wicks were first moistened and then positioned in the appropriate places. The electrophoresis tank was filled with barbitone buffer, and each compartment was filled with the substance. During the whole of this operation, the cellulose paper was submerged in the barbitone buffer for a minimum of five minutes. It was necessary to undertake a gradual immersion of the paper in order to avoid the creation of air bubbles and to ensure that the membrane was soaked in an equal manner. In between the membrane and the absorbent paper, there were two sheets of absorbent paper that were sandwiched for protection. For each of the samples, a tenth of a millilitre was placed into the well that was specifically allocated for such samples.

Initially, the applicator was submerged in the sample wells, and then the samples were transferred to the cellulose-acetate membrane at a distance of about two cm from one end of the membrane. After allowing the applicator tips to remain in contact with the membrane for a period of three seconds, the application would be completed. After that, the cellulose-acetate membrane was positioned in such a way that it was in contact with the buffer as well as the line that represented the cathode end. It was possible to do this by positioning the membrane such that it covered the bridge that was located between the two components of the tank. Twenty minutes were spent with the power supply attached and operating at a voltage range of 280-300 volts. This was done in order to achieve a detectable separation between the two components. It was also necessary to remove the cellulose-acetate membrane from the device, in addition to removing the power supply. Furthermore, the procedures for determining HbA2 levels are as follows.

CONCLUSION

Due to the low bacterial burden in specimens and various clinical symptoms, Extrapulmonary Mycobacterium tuberculosis (EPMTB) presents unique diagnostic problems. Based on the results of this research, it is clear that there is no perfect diagnostic procedure for all extrapulmonary samples. While traditional culture techniques are considered the gold standard, they may be somewhat time-consuming and may contribute to therapeutic delays.

Molecular methods, including PCR and GeneXpert MTB/RIF, are ideal in many clinical situations because of their sensitivity and speed of detection, which is particularly important in cases when a quick diagnosis is required. These approaches may be successful or ineffective, depending on the extrapulmonary specimen type and the resources that are available. To enhance patient outcomes and optimize diagnostic accuracy, an integrated diagnostic strategy combines molecular tests with microbiological and histopathological approaches. The kind of specimen, the availability of tests, the cost, and the turnaround time must all be taken into account when choosing an appropriate testing procedure. Early diagnosis and treatment of EPMTB may be improved by adapting the diagnostic approach to the specific clinical setting. This, in turn, will lead to better disease control.

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