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To Compare Molecular Methods with Conventional Diagnostic Methods of Tb Infection

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Abstract

In nations with poor to moderate incomes, tuberculosis is often regarded as the leading cause of mortality. In the recent past and in the current day, in addition to the increasing prevalence of tuberculosis (TB), particularly multidrug-resistant tuberculosis (MDR TB), and diabetes mellitus (DM), the association between the two illnesses has become a pressing public health issue . This connectivity is further an extra feeling in developing nations where tuberculosis is a native illness and an ascendancy of diabetes is noticed.

INTRODUCTION

A combination of case detection and treatment for tuberculosis is the most effective weapon in the fight against this ailment. In light of the negative influence that vaccinations have on the effectiveness of tuberculosis prevention, the option that is automatically ranked as the most effective means of limiting transmission is early detection and medical treatment. The sample microscopic inspection, radiography, and culture are the most essential instruments for detecting cases of tuberculosis [1]. As a result of its dependability, cost-effectiveness, and tranquility, direct microscopic observation has emerged as the approach that is accepted all over the globe. ZN staining, also known as Ziehl–Neelsen staining, is a procedure that is both quick and inexpensive for detecting AFB. Egg-based media, in particular Lowenstein–Jensen (LJ) media, are recognised as some of the most effective solid media for the isolation of mycobacterium tuberculosis (MTB). The process of culture is not only challenging, laborious, and taking a considerable amount of time (at least six to eight weeks), but it also requires specialized knowledge and competence. To be more specific, the acquisition of live bacteria is challenging and the time need is considerable in individuals who have been treated for tuberculosis. In this circumstance, conformation is often determined by the development of the culture and the biological properties of the culture, which are accomplished by the use of certain

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biochemical or molecular techniques [2]. In spite of this, the standard microbiological techniques continue to be the most important diagnostic tools for tuberculosis (TB), despite the fact that they have a poor sensitivity in direct testing and need a significant amount of time for culture procedures. In light of this, new diagnostic procedures that have been developed that have a higher yield, a wider range of sensitivity and specificity, and are affordable have been developed. These approaches have the potential to replace direct testing and culture [3].

The molecular diagnostic that is based on PCR is much quicker, more sensitive, and reduces the amount of time that is required for MTB detection and identification [4]. The old microbiological approaches have several limitations, which may be attributed to the fact that a disease like tuberculosis, which is a chronic and widespread condition, needs a diagnosis that is very prompt, sensitive, and final. Therefore, despite the fact that molecular techniques are the most promising approaches for diagnosing tuberculosis, their sensitivity is limited in samples that have negative sputum smear microscopy findings and in samples that are taken from extrapulmonary areas. However, in a few validation studies, the Line probe assay has been shown to have greater diagnostic accuracy for MTB identification in smear-positive patients and resistance to isoniazid and/or rifampin. Furthermore, the findings of the test may be obtained within one to two days [5] LPA is not a complete substitute for culture and DST (Drug Susceptibility Test) traditional procedures, despite the fact that it produces rapid results and has the same performance characteristics as other methods. It also has significant drawbacks. Despite the fact that the sensitivity of LPA in smear-negative samples is poor, it is necessary to do broader drug resistance testing using traditional DST. The LPA assay is a technically complex test that will demand significant laboratory resources and will necessitate the purchase of expensive equipment and reagents [6]. Within the scope of this research, our objective was to assess several techniques for diagnosing tuberculosis (TB) and to contrast the traditional techniques with the molecular testing techniques. For this reason, traditional techniques such as smear microscopy, culture and biochemical procedures, and molecular approaches such as LPA and spoligotyping are used for the purpose of detecting and identifying mycobacteria in a short amount of time. Through the use of molecular techniques, the primary objective is to facilitate the early treatment of the illness and to achieve more efficient management of it.

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MATERIALS AND METHODS

MATERIALS

Study objective

The objective of this study is to identify and compare the traditional techniques with the molecular approaches as they pertain to the diagnosis of tuberculosis (TB) using sputum samples directly.

Study design

Within the scope of this research, we conducted a prospective evaluation of every diagnostic approach among the participants in the study population. From 2013 to 2017, diabetic patients who were proven to have diabetes presented themselves at several diabetic clinics in Warangal, which is located in Telangana, India. The use of a questionnaire was used to study individuals ranging in age from one to ninety years old for the presence of symptoms that are indicative of tuberculosis. In the event that one or more of the following symptoms of tuberculosis are present, including an unexplained cough for a period of two weeks or longer, fever for a period of two weeks or longer, weight loss, and excessive sweating. Since these symptoms are present, the individuals in question are deemed to be potential tuberculosis suspects. Individuals suspected of having tuberculosis were recommended to undergo further testing by means of X-ray after the screening of their symptoms. Using a mix of radiographic, conventional, and molecular approaches in addition to the biochemical procedures was the last precedent for diagnosing tuberculosis when it was used. The microbiology lab at Sri Shivani College of Pharmacy receives samples of sputum from patients who are suspected of having tuberculosis. There, the samples are processed further. Various smear microscopy techniques, including as AFB, FM, and LED, were used to analyze the samples. Additionally, bacterial cultures were cultured on both solid and liquid media, which included the purification of sputum, neutralization, and inoculation onto both types of media. As a result of a macroscopic and microscopic examination of colonies on medium, it was determined that the cultures that were found to be positive from both types of media were of the MTB species. Isoniazid and rifampicin drug resistance mutations were then analyzed by polymerase chain reaction (PCR) in connection to its molecular genotyping, employing line probe assay platform, and spoligotyping. After that, they were further speciated and processed. The niacin test, the nitrate reductase test, and the catalase test were carried out at room temperature and at 68 degrees Celsius in order to prove that the MTB species was present.

Study population

In the current research, a total of two hundred individuals who were suspected of having tuberculosis and other lung diseases were collected. These patients might be of any gender or age group. Patients with diabetes mellitus who exhibited signs of tuberculosis, including complaints of cough that persisted for two weeks or longer, weight loss, fever, night sweats, and exhaustion, were included in the study, and sputum samples were taken from those patients.

Methodology

Chest X-ray screening

All of the diabetic patients who were eligible for the screening were given a chest X-ray, which was performed using digital X-ray equipment. This was done after the screening of their symptoms. A individual was believed to have tuberculosis (TB) if there was any abnormality in the lung fields or mediastinum during the first evaluation, which was merely for the purpose of screening. All of the X-ray pictures were saved digitally for future reference, and it was much more convenient to read them again. A further investigation was conducted on diabetic individuals who were suspected of having tuberculosis. The expectorate was analyzed by microscopy and culture. Persons who had a positive culture for tuberculosis and/or a positive sputum smear were considered to be a bacteriologically proven case of tuberculosis.

Sample collection and processing

In order to authenticate the chest X-rays for the purpose of sample collection, the symptoms and physical indicators will be taken into consideration. Under stringent aseptic conditions and using sterile containers, sputum samples (both early morning and spot sputum samples) of diabetic patients who have lung infections that are thought to be caused by tuberculosis are obtained by qualified researchers. These samples are then delivered to Sri Shivani College of Pharmacy, which is situated in Warangal. It was determined that a total of two hundred sputum samples were collected. Using an equivalent amount of 0.5 percent Nacetyl-L-cysteine (NALC) with 2% sodium hydroxide (NaOH) and 1.45 percent sodium citrate solution, the samples were digested and decontaminated [7]. This was done in accordance with a process that was published earlier by Petersburg. To ensure that the mixture was completely homogenized, it was left to remain at room temperature for fifteen to twenty minutes. After that, the adding of the phosphate buffer is carried out and well mixed. The item was centrifuged for at least fifteen

to twenty minutes at a speed of three thousand revolutions per minute. The sediment was resuspended in PBS after the supernatant was carefully discarded and resuspended it. Following that, the smears that were made from an aliquot of the sediment are used for the purposes of culture, DNA extraction, and microscopic examination.

RESULTS AND DISCUSSIONS

RESULTS

Conventional Methods

As of the 200 sputum samples gathered from people suspected of having tuberculosis, 113 were from males, 85 were from females, and two were from children. In all, there were 200 samples taken, and the findings of the chest X-ray showed that 55 of the sputum samples were positive. Of these, 30 were male, 25 were female, and there were no children discovered to be positive in the chest X-ray results. Sputum samples from thirty individuals were found to be positive for AFB, with seventeen of the suspects being male and thirteen of the suspects being female. On the basis of fluorescence staining and LED staining, 36 out of a total of 200 sputum samples were found to be positive for AFB. Twenty of the samples were male, and sixteen were female.

Smear Microscopy

Acid Fast Bacilli Microscopy

Bacilli that are acid fast may be used to diagnose tuberculosis. Slides that have been stained with carbolfuschin using ZN stain, which is resistant to decolorization that is caused by H2S04 and alcohol. Using either heating or alcohol fixation, fix the specimen smear onto the glass slide. Then, pour carbolfuschin over the smear, heat it gently until fumes form, and then wash it off with water from the glass slide. After pouring 20% sulfuric acid over the slide, the color of the slide changes to a pale pink. Cleanse your body with water. The methylene blue solution should then be washed with water once more. Give it some time to dry naturally, then inspect it with an oil immersion lens. In order to determine whether or not the smear produced positive results, the AFB staining procedure was used. Figure 20 depicts the slide as having a pinkish colored appearance.

Fluorescent and LED Microscopy

An sputum sample was obtained from a single site in the morning and placed in a sterile container. A purulent part of the yellow sputum was used to produce a smear, which was then applied on a bamboo stick that had been sterilized. It was then heat fixed by putting the slide

over a plate for three minutes, staining it using the auramine O staining process, and examining it using a fluorescence microscope. The smear was spread out uniformly, air-dried for fifteen minutes, and then studied. An appearance of gold may be seen on the slide.

Culture

Diagnosing pulmonary TB using culture is considered to be the gold standard approach.

Solid Media

LJ solid medium, which stands for Lowenstein-Jensen, was used to inoculate the homogenized samples. The development of Mycobacterium tuberculosis was accomplished with the help of L-J media, which is consisting of egg-based medium. Inoculation and incubation took place for a period of six to eight weeks for each and every TB-positive sample. It was determined that the cultures had grown by observing the morphology of the Mycobacterium colonies, which were described as rough, tough, and buff in color. The cultures were incubated at 37 degrees Celsius. The majority of countries with low incomes are the ones that utilize it the most.

Liquid Media

Homogenization of the bacterial suspension was achieved by the use of vortex shakeup, and the turbidity was adjusted in accordance with the tube using the McFarland no. 1 scale, which is 3.2 × 106 colony-forming units per milliliter. After diluting the bacterial suspension in Middle Brook 7H9 broth medium at a ratio of 1:20, the inoculum was produced that was suitable for use. It is necessary to utilize this diluted solution, which is 100 microliters in volume, to inoculate in order to evaluate the drug activity. The usual amount of time required to identify the development of mycobacteria is between 12 and 16 days. This is a fast growth of mycobacteria.

DISCUSSION

Since many years ago, tuberculosis has continued to be a significant health concern that threatens mankind, despite the fact that it is a disease that is understood and can be treated. Comparative and analytical research was conducted in this study between the traditional techniques and the molecular approaches for diagnosing tuberculosis. The PCR-based molecular technique for tuberculosis demonstrated the highest diagnostic yield, followed by smear microscopy of AFB, LED, and FM, culture, and differential staining method. One of the

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earliest methods for diagnosing tuberculosis is the sputum smear microscopy, which is not only straightforward but also quick and economical. The specificity and sensitivity of this approach are poor, and the results of the AFB slide observation are reliant on human potential, the availability of the laboratory, and the support of technical personnel [8]. Due to the fact that the culture techniques need a significant amount of time, clinical expertise and evidence such as radiological diagnosis continue to be the key components that are used in the process of the patient being medicated with anti-tubercular medicine [9].

When it comes to the beginning of therapy and the management of sickness, one of the most significant factors is the rapid and precise identification of pathogens, as well as the characterization of their medication vulnerabilities. Consequential research and operational preferences have been carried out [10] in order to facilitate the detection of mycobacterium tuberculosis and treatment resistance in a more expedient manner. At the moment, a great number of molecular instruments are prepared for use. These techniques, in general, detect the DNA or RNA of MTB and amplify it using polymerase chain reaction. However, there are a few exceptions to this rule, which recognize divergency and modifications to differentiate MTB, bacterial species, and the diversity of MTB clusters. A speedy and reliable detection of tuberculosis might also be achieved by the use of molecular technologies, as well as genotyping, resistance to rifampin, and resistance to isoniazid. These procedures have the advantage over culture-based methods in that they are much quicker and reduce the amount of time that is wasted on delayed diagnosis. Sputum samples might be used for PCR-based MTB identification [11]. Such an identification could be conducted.

According to the findings of a research conducted by Siddiquiet al. [12], the positive rate of ZN staining, LJ culture media, and PCR techniques is 5%, 15%, and 70%, respectively. In one of the most recent research that was carried out by Sharma et al. [13], it was discovered that polymerase chain reaction (PCR) is an accurate method for diagnosing paucibacillary disorders such as EPTB. In the course of their research, the total sensitivities of microscopy, culture, and PCR were 7.33 percent, 11.33 percent, and 74.6 percent, respectively. On the other hand, the samples that were found to be positive by ZN smear and LJ culture were also found to have positive findings when they were tested with PCR in this particular investigation. The staining

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yield, which was 36 samples, was much greater than the culture positivity [14] yield, which was significantly lower than the PCR yield.

In one of the tests that were carried out in Bangladesh, the results showed a PCR yield of 68.9 percent.

A high level of clinical uncertainty about the patient is the starting point for the tuberculosis diagnosis process, which is supported by the use of a variety of diagnostic procedures. The sole quick diagnostic approach for presumptive tuberculosis is the smear examination, and the final positive laboratory diagnostic method is the culture method, which is considered the gold standard. For molecular techniques, however, its decreased sensitivity in identifying MTBC in clinical specimens has been tackled. The area of tuberculosis has been revolutionized by molecular methods, including as the LPA and spoligotyping, which have allowed for more precise and speedy diagnosis, improved sensitivity and specificity, and the identification of species. However, in the context of the diagnosis of tuberculosis in the current day, molecular approaches represent a benefit, despite the fact that they are much more expensive than the conventional ways of cultivating solid or liquid media. Chest X-rays and smear microscopy are the methods of choice for the first evaluation of patients suspected of having tuberculosis in the current research. This is due to the fact that smear microscopy has a high sensitivity (even in a single sample), because it is simple to conduct, and because it is less expensive. The molecular approaches based on PCR have been proven as the most sensitive way for diagnosing tuberculosis. These methods are comparable to smear microscopy, and they have the advantages of providing quicker results and contemporaneous detection of MTBs. However, they are also expected to be more costly [15]. The molecular approaches have nonetheless begun to be favored over the traditional methods for the speedier diagnosis of pulmonary tuberculosis, despite the fact that their sensitivity is lower than acceptable in samples with negative culture findings even if they have been used. Molecular approaches, on the other hand, provide findings in a shorter amount of time than culture methods when it comes to detecting MTB. The integrated examination of conventional clinical, radiographic, and microbiological observations does not offer the diagnosis. Immediately, there are no alternative approaches that are more competent than the one that is provided [16]. The relatively limited number of samples and the lack of a sufficient number of positive samples are the most significant constraints of our research. These limitations make it difficult to assess the effectiveness of the approaches in finding resistance.

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