ORIGINAL RESEARCH PAPER

Pathology

EVALUATION OF DIAGNOSTIC ROLE OF XENEXPERT IN PULMONARY TUBERCULOSIS.

KEY WORDS: Tuberculosis, Genexpert, TB, Mycobacterium tuberculosis, Sensitive.

Dr. Versha Prasad Assistant Professor, School Of Health Sciences C.S.J.M. University, Kanpur.

Kapil Kumar Singh Research Student, M.Sc. MLT, Medical Laboratory Technology, School Of Health Sciences, C.S.J.M. University Kanpur.

ABSTRACT

The aim of this study was prevalence of GeneXpert in Pulmonary Tuberculosis.Tuberculosis (TB) is a communicable disease and a major cause of ill health. It is one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS). The diagnosis of Mycobacterium tuberculosis (MTB) lung infection (pulmonary tuberculosis) is historically based on identification of the bacterium by direct examination or by culture of a respiratory sample. Since 2010, a specific polymerase chain reaction (PCR) assay, known as GeneXpert, has been used worldwide.The GeneXpert MTB/RIF assay is a new diagnostic test that depends on a real-time PCR analysis of the sample for diagnosis of pulmonary tuberculosis (TB).This study includes 100 suspected cases of tuberculosis who visited the Department of Tuberculosis and Respiratory Diseases, GSVM Medical College for diagnosis and treatment. Early morning sputum samples were collected in a sterile, clean, dry and wide mouth container. Sputum samples were analysed by using GeneXpert. GeneXpert is an rapid and automatic nucleic acid amplification test (NAAT) for Mycobacterium tuberculosis.Examination of 100 patients was done with the help of GeneXpert, age group between 16-85 years. Out of 100 patients, there were 69 male patients and 31 female patients. Out of 100 patients, there were 32 positive and 68 negative for Mycobacterium tuberculosis. Out of 32 positive patients, there were 19 male patients and 13 female patients. Genexpert confirmed the clinical diagnosis in 32% cases only, while 68% cases were negative.Genexpert is a sensitive, rapid, accurate, easy to carry out and important diagnostic tool especially in early and doubtful cases of pulmonary tuberculosis.The GeneXpert assay can be used as a gold standard to diagnose TB. As it is sensitive and specific for diagnosing smear-negative pulmonary TB, the assay is faster than culture.

INTRODUCTION

Tuberculosis is one of the oldest known human pathogens. The disease can be traced back through historical references and evidence of infections in human remains from some of the most ancient civilizations. Evidence of tuberculosis infections has been found in the necropoli of Ancient Egypt, Neolithic skeletons from burial sites in Europe, and mummies excavated from the Andes Mountains in South America but statistical models have estimated that the Mycobacterium tuberculosis complex may have evolved 40,000 years ago around the same time that human populations are thought to have begun to expand and migrate out of Africa.[1,2]Koch (1882) isolated the mammalian tubercle bacillus and proved its causative role in tuberculosis by satisfying certain postulates (known as Koch's postulates).Tuberculosis in human was subsequently shown to be caused by two types of the bacillus: the human and bovine types, designated Mycobacterium tuberculosis and M. bovis, respectively. [3]Tuberculosis (TB) is caused by a bacterium called *Mycobacterium tuberculosis*. The bacteria usually attack the lungs, but TB bacteria can attack any part of the body such as the kidney, spine, and brain. Not everyone infected with TB bacteria becomes sick. As a result, two TB-related conditions exist: latent TB infection (LTBI) and TB disease. If not treated properly, TB disease can be fatal. [4,5]Every year, 10 million people fall ill with tuberculosis (TB). Despite being a preventable and curable disease, 1.5 million people die from TB each year – making it the world's top infectious killer. TB is the leading cause of death of people with HIV and also a major contributor to antimicrobial resistance. Most of the people who fall ill with TB live in low- and middle-income countries, but TB is present all over the world. About half of all people with TB can be found in 8 countries: Bangladesh, China, India, Indonesia, Nigeria, Pakistan, Philippines and South Africa.[6,7]About a quarter of the global population is estimated to have been infected with TB bacteria, but most people will not go on to develop TB disease and some will clear the infection. Those who are infected but not (yet) ill with the disease cannot transmit it.[8,9]

People infected with TB bacteria have a 5–10% lifetime risk of falling ill with TB. Those with compromised immune systems, such as people living with HIV, malnutrition or diabetes, or people who use tobacco, have a higher risk of falling ill.[10,11]When a person breathes in TB bacteria, the bacteria can settle in the luns and begin to grow. From there, they can move through the blood to other parts of the body, such as the kidney, spine, and brain.[12,13]

The probability of transmission from one person to another depends upon several factors, including the number of infectious droplets expelled by the carrier, the effectiveness of ventilation, the duration of exposure, the virulence of the M. tuberculosis strain, the level of immunity in the uninfected person, and others. The cascade of person-to-person spread can be circumvented by segregating those with active ("overt") TB and putting them on anti-TB drug regimens. After about two weeks of effective treatment, subjects with nonresistant active infections generally do not remain contagious to others. If someone does become infected, it typically takes three to four weeks before the newly infected person becomes infectious enough to transmit the disease to others.[14,15]M. tuberculosis is a straight or slightly curved rod, about 3 μm x 0.3 μm in size, occurring singly, in pairs or as small clumps. The size depends on conditions of growth.[16,17] Long, filamentous, club-shaped and branching forms may sometimes be seen. Electron micrographs of thin sections show a thick cell wall composed of three layers enclosing a trilaminar plasma membrane. Spheroplasts and L forms are formed when grown in the presence of lysozyme.[18]

The Xpert MTB/RIF Assay, performed on the GeneXpert Instrument Systems, is a qualitative, nested real-time polymerase chain reaction (PCR) in vitro diagnostic test for the detection of Mycobacterium tuberculosis complex DNA in raw sputum or concentrated sputum sediment prepared from induced or expectorated sputum. In specimens where Mycobacterium tuberculosis complex (MTB-complex) is detected, the Xpert MTB/RIF Assay also detects the rifampin-resistance associated mutations of the rpoB gene.[19]This test is intended as an aid in the diagnosis of pulmonary tuberculosis when used in conjunction with clinical and other laboratory findings.[20]

MATERIALS & METHODS

A retrospective and observational study was carried out among patient of Pulmonary Tuberculosis enrolled in the department of Pathology, Microbiology& collaboration with Respiratory Medicine department&DOTs Centre at G.S.V.M Medical college, Kanpur.100 suspected cases of patient of Pulmonary Tuberculosis enrolled at DOTs Centre were included in the study age group both genders attending the Respiratory ward&DOTs Centre of the hospital for last 6 months have been included in this study.

Inclusion Criteria-

Suspected patients of Pulmonary tuberculosis of all ages, patients who have negative for AFB Staining.

Exclusion Criteria-

Patients diagnosed with Pulmonary Tuberculosis were excluded from the study, patient with significant co-morbid illness, terminally ill patient.

Sample Collection:

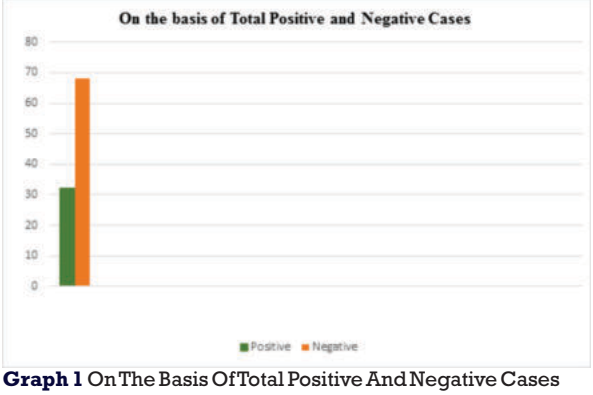
Morning sputum sample are collected in sterile plastic Container for collecting sputum.

OBSERVATION AND RESULT

In the present study, 100 confirmed cases of patient of Pulmonary Tuberculosis enrolled at DOTs Centre were included in the study age group both genders attending the Respiratory ward &DOTs Centre of the hospital Out of 100 patients, there were 69 male patients and 31 female patients. Out of 100 patients, there were 32 positive and 68 negative for Mycobacterium tuberculosis. Out of 32 positive patients, there were 19 male patients and 13 female patients. Genexpert confirmed the clinical diagnosis in 32% cases only, while 68% cases were negative.

Table No 1 – On The Basis Of Total Positive And Negative Cases

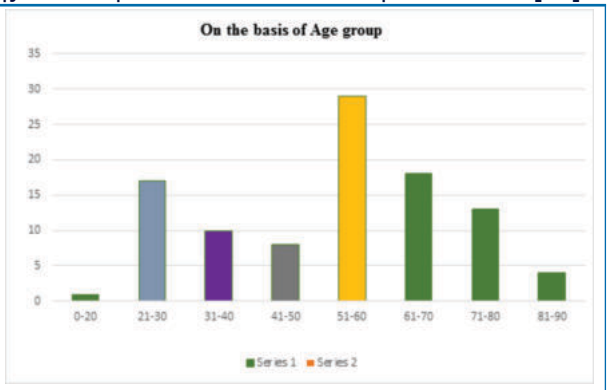
Total Cases	No. of Positive cases	No. of Negative cases
100	32	68



Graph 1 On The Basis Of Total Positive And Negative Cases

Table No 2 – On The Basis Of Age Group No. Of Suspected Cases Of Pulmonary Tuberculosis

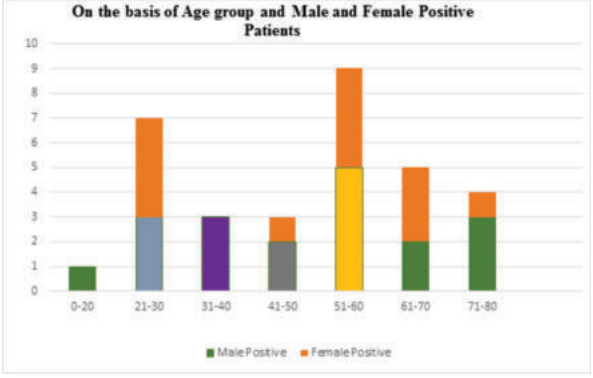
AGE groups	No. of cases = 100	Percentage
10-20	01	01%
21-30	17	17%
31-40	10	10%
41-50	08	08%
51-60	29	29%
61-70	18	18%
71-80	13	13%
81-90	04	04%



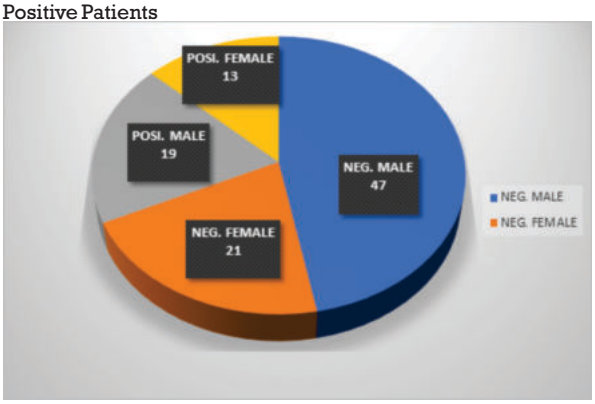
Graph 2 On the basis of Age group No. of Suspected cases of pulmonary tuberculosis

Table No 3 – On The Basis Of Age Group And Male And Female Positive Patients

AGE groups	No. of cases Positive Male	No. of cases Positive Female
10-20	01	00
21-30	03	04
31-40	03	00
41-50	02	01
51-60	05	04
61-70	02	03
71-80	03	01



Graph 3 On the basis of Age group and Male and Female Positive Patients



Graph 4 Male and Female Positive and Negative Patients

DISCUSSION

In the present study, 100 suspected cases of patient of Pulmonary Tuberculosis enrolled at DOTs Centre&) who visited the Department of Microbiology GSVM Medical College for diagnosiswere included in the study age group both genders attending the Respiratory ward &DOTs Centre of the hospital Out of 100 patients, there were 69 male patients and 31 female patients. Out of 100 patients, there were 32 positive and 68 negative for Mycobacterium tuberculosis. Out of 32 positive patients, there were 19 male patients and 13

female patients. Early morning sputum samples were collected in a sterile, clean, dry and wide mouth container. Sputum samples were analysed by using GeneXpert. Genexpert confirmed the clinical diagnosis in 32% cases only, while 68% cases were negative.

CONCLUSION

Genexpert is a sensitive, rapid, accurate, easy to carry out and important diagnostic tool especially in early and doubtful cases of pulmonary tuberculosis. The GeneXpert assay can be used as a gold standard to diagnose TB. As it is sensitive and specific for diagnosing smear-negative pulmonary TB, the assay is faster than culture.

REFERENCES

1. https://www.who.int/health-topics/tuberculosis#tab=tab_1
2. https://www.cdc.gov/tb/topic/basics/default.html.
3. https://en.wikipedia.org/wiki/Tuberculosis.
4. Costantini L, Marando M, Gianella P. Long-Term GeneXpert Positivity after Treatment for Pulmonary Tuberculosis. Eur J Case Rep Intern Med. 2020 Jul 6;7(10):001737. doi: 10.12890/2020_001737. PMID: 33083351; PMCID: PMC7546557.
5. Sedky, Mohamed; Wakil, Ismail A.; Rashed, Mohamed; Salama, Ahmed. The role of genexpert in diagnosis of sputum-negative pulmonary tuberculosis. The Egyptian Journal of Chest Diseases and Tuberculosis 67(4):p 419-426, Oct–Dec 2018. | DOI: 10.4103/ejcdt.ejcdt_57_18
6. Madeleine G. Moule, Cirillo Jeffrey D. "Mycobacterium tuberculosis Dissemination Plays a Critical Role in Pathogenesis," 2020 Feb 25;10:65. doi: 10.3389/fcimb.2020.00065. PMID: 32161724; PMCID: PMC7053427.
7. Campbell IA, Bah-Sow O. "Pulmonary tuberculosis: diagnosis and treatment." BMJ. 2006 May 20;332(7551):1194-7. doi: 10.1136/bmj.332.7551.1194. PMID: 16709993; PMCID: PMC1463969.
8. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev. 2014 Jan 21;2014(1):CD009593. doi: 10.1002/14651858.CD009593.pub3. Update in: Cochrane Database Syst Rev. 2019 Jun 07;6:CD009593. PMID: 24448973; PMCID: PMC4470349.
9. Akanbi MO, Achenbach C, Taiwo B, Idoko J, Ani A, Isa Y, Agbaji O, Ukoli C, Akande P, Maiga M, Murphy RL. Evaluation of gene xpert for routine diagnosis of HIV-associated tuberculosis in Nigeria: A prospective cohort study. BMC Pulm Med. 2017 May 30;17(1):87. doi: 10.1186/s12890-017-0430- 6. PMID: 28558662; PMCID: PMC5450144.
10. Kaso AW, Hailu A. Costs and cost-effectiveness of Gene Xpert compared to smear microscopy for the diagnosis of pulmonary tuberculosis using real-world data from Arsi zone, Ethiopia. PLoS One. 2021 Oct 25;16(10):e0259056. doi: 10.1371/journal.pone.0259056. PMID: 34695153; PMCID: PMC8544827.
11. Lawn SD, Zumla AI. Diagnosis of extrapulmonary tuberculosis using the Xpert(®) MTB/RIF assay. Expert Rev Anti Infect Ther. 2012 Jun;10(6):631-5. doi: 10.1586/eri.12.43. PMID: 22734954; PMCID: PMC3605769.
12. Pinto MFT, Steffen R, Entringer A, Costa ACCD, Trajman A. [Budget impact of the incorporation of GeneXpert MTB/RIF for diagnosis of pulmonary tuberculosis from the perspective of the Brazilian Unified National Health System, Brazil, 2013-2017]. Cadernos de SaudePublica. 2017 Oct;33(9):e00214515. DOI: 10.1590/0102-311x00214515. PMID: 29019524.
13. Ben J. Marais, Robert P. Gie, H. Simon Schaaf, NuldaBeyers, Peter R. Donald, Jeff R. Starke. (2006) Childhood Pulmonary Tuberculosis. American Journal of Respiratory and Critical Care Medicine **173**:10, 1078-1090.
14. Morris CD. Pulmonary tuberculosis in the elderly: a different disease? Thorax. 1990 Dec;45(12):912-3. doi: 10.1136/thx.45.12.912. PMID: 2281421; PMCID: PMC462835.
15. CROFTON J. Chemotherapy of pulmonary tuberculosis. Br Med J. 1959 Jun 27;1(5138):1610-4. doi: 10.1136/bmj.1.5138.1610. PMID: 13662631; PMCID: PMC1993570.
16. Sudre P, ten Dam G, Kochi A. Tuberculosis: a global overview of the situation today. Bull World Health Organ. 1992;70(2):149-59. PMID: 1600578; PMCID: PMC2393290.
17. Shrestha P, Khanal H, Dahal P, Dongol P. Programmatic Impact of Implementing GeneXpert MTB/ RIF Assay for the Detection of Mycobacterium Tuberculosis in Respiratory Specimens from Pulmonary Tuberculosis Suspected Patients in Resource Limited Laboratory Settings of Eastern Nepal. Open Microbiol J. 2018 Feb 28;12:9-17. doi: 10.2174/1874285801812010009. PMID: 29541267; PMCID: PMC5842389.
18. Agrawal M, Bajaj A, Bhatia V, Dutt S. Comparative Study of GeneXpert with ZN Stain and Culture in Samples of Suspected Pulmonary Tuberculosis. J Clin Diagn Res. 2016 May;10(5):DC09-12. doi: 10.7860/JCDR/2016/18837.7755. Epub 2016 May 1. PMID: 27437212; PMCID: PMC4948388.
19. Ikuabe PO, Ebuenyi ID. Prevalence of rifampicin resistance by automated Genexpert rifampicin assay in patients with pulmonary tuberculosis in Yenagoa, Nigeria. Pan Afr Med J. 2018 Apr 6;29:204. doi: 10.11604/pamj.2018.29.204.14579. PMID: 30100958; PMCID: PMC6080957.
20. MoussaHSh, Bayoumi FS, Ali AM. Evaluation of GeneXpert MTB/RIF assay for direct diagnosis of pulmonary tuberculosis. Saudi Med J. 2016 Oct;37(10):1076-81. doi: 10.15537/smj.2016.10.14998. PMID: 27652357; PMCID: PMC5075370.