

COMPARISON OF GENE EXPERT[®] AND TUBERCULIN TEST FOR THE DIAGNOSIS OF CHILDHOOD TUBERCULOSIS

Jamal Anwer,¹ Humayun Iqbal Khan,¹ Mubarak Ali,¹ Kifayat Niazi,¹ Ghulam Qasim

ABSTRACT

Background: Tuberculosis is a lethal communicable disease, and at times its diagnosis is difficult to establish. **Objective:** To compare the results of Gene Expert[®] to that of tuberculin test for the diagnosis of childhood tuberculosis. **Patients and Methods:** Study design: Cross-Sectional Analytical study. Place and duration of study: Department of Pediatrics, Sheikh Zayed Medical College & Hospital, Rahim Yar Khan from 1st November, 2012 to 31st July, 2013. 92 patients of tuberculosis were included strictly, following the inclusion and exclusion criteria. Diagnosis of tuberculosis was on the basis of Kenneth Jones criteria. Beside other investigations Gene Expert[®] and Tuberculin test were done and their results were compared. The data was entered and analyzed by SPSS version 15. **Results:** Out of 92 patients, 80 (86.9%) were reported positive by Gene Expert whereas 56 (60.8%) were reported positive by Tuberculin test. Overall 52 (56.5%) were found positive by both Gene Expert & Tuberculin test, whereas 8 (8.6%) were found negative by both Gene Expert and Tuberculin test. Out of 80 who were found positive by Gene Expert, 28(35%) were reported negative by Tuberculin test while out of 12 who were reported negative by Gene Expert, 4 (33.3%) were positive by Tuberculin test. **Conclusion:** In our study, we found that both Gene Expert[®] and Tuberculin test are effective diagnostic tools for tuberculosis. However, in several cases Gene Expert[®] is more effective than Tuberculin test.

Key words: Gene Expert[®], Tuberculin test by Purified Protein Derivative, Childhood tuberculosis.

INTRODUCTION

Tuberculosis is a common, and in many cases lethal, infectious disease caused by various strains of mycobacterium, usually *Mycobacterium tuberculosis*. Tuberculosis typically attacks the lungs, but can also affect other parts of the body.^{1,2} Most infections are asymptomatic and latent, but about one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50%.³ One third of the world's population is thought to have been infected with *M. tuberculosis*, with new infections occurring in about 1% of the population each year. In 2007, there were an estimated 13.7 million chronic active cases globally, while in 2011, there were an estimated 8.7 million new cases and 1.42 million associated deaths, mostly occurring in developing countries. Of these 990,000 were among HIV negative people and there were a further 430,000 deaths among HIV positive

people. In 2009 there were almost 10 million children who were orphans as a result of at least one of their parents dying of TB.^{2,4} The distribution of tuberculosis is not uniform across the globe; about 80% of the population is Asian (including Pakistan) and African who are positive in tuberculin test, while only 5-10% of the United States population tests positive.^{2,5} More people in the developing world contract tuberculosis because of compromised immunity, largely due to high rates of HIV infection and the corresponding development of AIDS. Tuberculosis (TB) is one of the major public health problems in Pakistan. Pakistan ranks fifth amongst TB high-burden countries worldwide. It accounts for 61% of the TB burden in the WHO Eastern Mediterranean Region. In Pakistan there are around 620,000 TB patients in the country. Every year, 410,000 new people develop TB with an estimated incidence of 231/100,000 population. Pakistan is the world's fourth highest multidrug-resistant tuberculosis (MDR-TB) burden country.^{3,6}

Diagnosis of active TB relies on clinical features and investigations e.g. radiology (commonly chest X-rays), as well as microscopic examination and microbiological culture of body fluids. Diagnosis of latent TB relies on the tuberculin skin test (TST) and/or blood tests.^{5,7} Tuberculin is a glycerol extract of the tubercle bacillus. Purified protein derivative

1. Department of Paediatrics, Sheikh Zayed Medical College/Hospital, Rahim Yar Khan, University of Health Sciences Lahore.

Correspondence: Dr. Jamal Anwer, Assistant Professor, Department of Paediatrics, Sheikh Zayed Medical College/Hospital, Rahim Yar Khan.

Email: drjamal.anwer@yahoo.com

Phone: 0300-7829858

(PPD) tuberculin is a precipitate of species-nonspecific molecules obtained from filtrates of sterilized, concentrated cultures. Newer technique for the diagnosis of TB includes Gene Expert. The Xpert MTB/RIF is a cartridge-based, automated diagnostic test that can identify *Mycobacterium tuberculosis* (MTB) and resistance to rifampicin (RIF) through Polymerase Chain Reaction.^{4,8}

Treatment of tuberculosis is difficult and requires administration of multiple antibiotics over a long period of time. Social contacts are also screened and treated if necessary. Antibiotic resistance is a growing problem in multiple drug-resistant tuberculosis (MDR-TB) infections. Prevention relies on screening programs and vaccination with the Bacillus Calmette-Guérin vaccine^{1,9}

Tuberculin skin test is a conventional test for the diagnosis of tuberculosis. However, false positive and false negative results are very common. Moreover resistance of antituberculous therapy especially rifampicin and isoniazid is very troublesome.¹⁰ Gene Expert is very effective modern technology for the diagnosis of tuberculosis and isolation of rifampicin/isoniazid resistance. We conducted this study for comparing Gene Expert and tuberculin test as a diagnostic tool, for childhood tuberculosis

PATIENTS AND METHODS

This cross-sectional study was conducted from 1st November, 2012 to 31st July, 2013. The study population was based on a total of 92 patients with the diagnosis of tuberculosis. They were all admitted in pediatric Unit, Sheikh Zayed Hospital, Rahim Yar Khan. Inclusion Criteria: In the study we included children below 14 years of age suspected to be suffering from tuberculous meningitis, pulmonary tuberculosis, tuberculous lymphadenitis or abdominal tuberculosis. Patients not fulfilling the above criteria were excluded. The data was entered and analyzed in SPSS version 15. We used Modified Kenneth Jones criteria for the diagnosis of tuberculosis with the exclusion of one point of tuberculin test.

According to this scoring system, 7 or more points indicate unquestionable TB; 5-6 points indicate probable TB, therapy may be justified; 3-4 points

indicate that further investigations are needed.

Samples (sputum/gastric lavage/CSF) were sent for Gene Expert[®] available in pathology department.

The *Xpert MTB/RIF* assay is a fully automated molecular diagnostic test for TB disease. It can simultaneously detect *Mycobacterium tuberculosis* (MTB) complex DNA and mutations associated with rifampicin (RIF) resistance directly from sputum specimens in less than 2 hours, and it minimizes staff manipulation and biosafety risk.^{9,10,11} *Xpert* can detect TB, including MDR-TB, in less than 2 hours, potentially reducing the time to diagnose and treat TB. In December 2010, the World Health Organization (WHO) endorsed the Xpert MTB/RIF for use in TB endemic countries and declared it a major milestone for global TB diagnosis.⁷

PPD for Tuberculin test is available in the department through TB DOT programme. It was given in standard dose on anterior aspect of forearm. The reading was taken on 2nd or 3rd day. The results were declared positive or negative on standard guidelines.

Tuberculin test Procedure: A standard dose is 5 tuberculin units is injected intradermally (between the layers of dermis) and read 48 to 72 hours later.¹ This intradermal injection is termed the Mantoux technique.

A person who has been exposed to the bacteria is expected to mount an immune response in the skin containing the bacterial proteins. The reaction is read by measuring the diameter of induration (palpable raised, hardened area) across the forearm (perpendicular to the long axis) in millimeters. If there is no induration, the result should be recorded as "0 mm". Erythema (redness) should not be measured.

Classification of tuberculin reaction: The results of this test must be interpreted carefully. A positive result indicates TB exposure.⁴

5 mm or more is positive in an HIV-positive person, persons with recent contacts with a TB patient, persons with nodular or fibrotic changes on chest X-ray consistent with old healed TB, patients with organ transplants, and other immunosuppressed patients

10 mm or more is positive in recent arrivals (less than five years) from high-prevalence countries, injection drug users, residents and employees of high-risk congregate settings (e.g. prisons, nursing homes,

hospitals, homeless shelters, etc) , mycobacteriology laboratory personnel, persons with clinical conditions that place them at high risk (e.g., diabetes, prolonged corticosteroid therapy, leukemia, end-stage renal disease, chronic malabsorption syndromes, low body weight, etc.), children less than four years of age, or children and adolescents exposed to adults in high-risk categories. 15 mm or more is positive in persons with no known risk factors for TB

False negative result: Immunologically compromised, especially those with HIV and low CD4 T cell counts. Steroid use, malnutrition and sarcoidosis can also lead to false-negative results.⁶

RESULTS

Most of the patients in our study were male 57 (62%). The number of patients in the age group 5-10 years was 39 (42.4%) while 31(34%) in 1-5 years and 22 (24%) in 10-14 years. Out of total 92 patients, 18 (19.5%) fathers and 29 (31.5%) mothers were illiterate. 72.8% of patients belonged to low socioeconomic status. Regarding fathers' ethnicity most of the patients i.e. 38 (41.3%) were Saraiki. Out of 92 patients, 80 (86.9%) were reported positive by Gene Expert whereas 56 (60.8%) were reported positive by Tuberculin test. Overall 52 (56.5%) were found positive by both Gene Expert & Tuberculin test, whereas 8 (8.6%) were found negative by both Gene Expert and Tuberculin test. Out of 80 who were positive by Gene Expert, 28 (35%) were reported negative by Tuberculin test while out of 12 who were reported negative by Gene Expert, 4 (33.3%) were positive by Tuberculin test. (Table: I)

Table I: Gene Expert and Tuberculin test results

Tuberculin test	Gene Expert		
	Test +ve	Test -ve	Total
Positive	52	04	56
Negative	28	08	36
Total	80	12	92

DISCUSSION

Tuberculosis (TB) remains a major global health problem. It causes ill-health among millions of people each year and ranks as the second leading

cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV).^{2,12} The World Health Organization (WHO) declared TB a global public health emergency in 1993. Starting in the mid-1990s, efforts to improve TB care and control intensified at national and international levels.²

Diagnosis of TB relies on clinical features and investigations, as well as microscopic examination and microbiological culture of body fluids. Diagnosis of latent TB relies on the tuberculin skin test (TST) and/or blood tests. Tuberculin test is a classical tool for the diagnosis of tuberculosis.^{2,13} However, its positivity or negativity is doubtful in some cases such as malnutrition, immune deficiency etc. We have used new technology of molecular genetics for the detection of Mycobacterium tuberculosis. Gene Expert[®] is an automated diagnostic test that can identify Mycobacterium tuberculosis.^{10,11,14} The Xpert MTB/RIF, a new rapid molecular test that can diagnose TB and rifampicin-resistant TB within hours, has been impressive. Between its endorsement by WHO in December 2010 and the end of June 2012, 1.1 million test cartridges were procured in 67 (46%) of the 145 countries eligible to purchase them at concessional prices. Acceleration in uptake is still needed to realize the full potential of the technology.^{2,15,16}

In our eight months study the patient who were strong candidate of tuberculosis whether pulmonary, GI, lymph nodes or CNS were investigated by both Tuberculin test and Gene Expert[®]. In our study, we included 92 patients strictly following the inclusion criteria. These results showed that 52 patients have both Gene Expert[®] and tuberculin test positive, and when we started ATT to these patients, they responded well. 28 patients have only Gene Expert positive and PPD negative. These patients favored all the inclusion criteria, so ATT started and they responded and got better. 4 patients have only Tuberculin test positive. They also favored all the inclusion criteria, so ATT started and they responded and got better. 8 patients have both Gene Expert and Tuberculin test negative. In these patients although inclusion criteria was also followed but in some points they were deficient of the criteria. Their diagnosis was revised and investigated. In a study by Blackmore R, described Expert a valuable test for diagnosis of tuberculosis.¹⁵

Hillemann D, Weizenegger et al¹⁷ and Huang, Chen,

Kuo et al¹⁸ evaluated the use of the Genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in mycobacterium tuberculosis complex isolates.^{15,16} Makinen J, Marttila H. J, Marjamaki M et al used the DNA line probe assays for detection of mycobacterium tuberculosis and found it to be very useful.¹⁹ Moure R, found that the rapid detection of mycobacterium tuberculosis complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method to be very effective.²⁰ Raja and E.A Khan concluded in their studies that PCR method is very useful for the detection of Mycobacterium Tuberculosis.^{21,22} Nicol et al,²³ made a comparison between ELISPOT and the tuberculin skin test in which some children with active TB resulting in negative T-SPOT-TB. These suggested that the test may have impaired sensitivity for very young children, for whom it should not be used to exclude the possibility of active tuberculosis.²¹ As Gene expert is a new modality of diagnosis and is being used only for last 2 years, comparison of results with other diagnostic tests is limited especially from national source.

CONCLUSION

Before the Gene Expert[®] we have conventional diagnostic tools for tuberculosis. In our study we used Gene Expert[®] and compared the results with that of tuberculin test. In our study, we conclude that Gene Expert is reliable as compared to tuberculin test. In SZMC Hospital, RY Khan, the use of Gene Expert[®] is found to be more helpful for the diagnosis of tuberculosis especially considering its utility as for as short time is concerned.

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