https://doi.org/10.29309/TPMJ/2024.31.12.8323

The Professional Medical Journal
www.theprofesional.com
2024, Volume, 31 Issue, 12

ORIGINAL ARTICLE

Comparison study of genexpert MTB / RIF assay versus sputum smear microscopy results in presumptive tuberculosis cases in district Ghotki, Sindh.

Rao Aftab Ahmed[1], Seema Qazi[2], Uzma Riaz[3], Miss Faiqa Memon[4], Hanna Khair Tunio[5], Sana Aqeel[6], Masood Ahmed Unar[7]

Article Citation: Ahmed RA, Qazi S, Riaz U, Memon MF, Tunio HK, Aqeel S, Unar MA. Comparison study of genexpert MTB / RIF assay versus sputum smear microscopy results in presumptive tuberculosis cases in district Ghotki, Sindh. Professional Med J 2024; 31(12):1690-1696. https://doi.org/10.29309/TPMJ/2024.31.12.8323

ABSTRACT… Objective: To evaluate the performance of GeneXpert MTB/RIF assay by comparing its results with sputum smear microscopy. **Study Design:** Comparative Cross-Sectional Study. **Setting:** Department of Outpatient (OPD), Taluka and District Head Quarter Hospitals of District Ghotki Sindh. **Period:** October 2022 to 2023. **Methods:** In this study 350 Participants were included and all sputum specimens were tested for both above mentioned diagnostic tools that are sputum smear microscopy and GeneXpert MTB/RIF assay during the period October 2022 to April 2023. All details regarding the study such as Demographic details, sputum smear microscopy results, and GeneXpert MTB/RIF assay test results of patients were collected. A case of pulmonary tuberculosis (PTB) was considered to be a patient having mycobacterium tuberculosis detected in sputum by GeneXpert MTB/RIF assay. **Results:** In this study, 61 cases (17.4%) out of 350 participants, were detected in GeneXpert MTB/ RIF assay and 35 cases (10%) out of 350 participants were positive in sputum smear microscopy. Male ratio is slightly higher than female among GeneXpert positive cases. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy sputum smear microscopy were 57%, 100%, 100%, 91.7%, and 92.5%, respectively, when GeneXpert test is taken as reference test. **Conclusion:** Sputum smear microscopy is as specific as GeneXpert but less sensitive test for the diagnosis of pulmonary tuberculosis than GeneXpert MTB/RIF assay.

Key words: Gene Xpert MTB/RIF Assay, MDR, Sputum Smear Microscopy, Tuberculosis.

INTRODUCTION

Tuberculosis (TB), with high prevalence is a global health problem and remained a major health issue since ancient times.[1] It is a severe air borne contagious disease, primarily affects the lungs (pulmonary TB), characterized by cough, more than 14 days and bloody sputum, decrease in weight, loss of appetite, tiredness and fatigue, sweating in -nights, Evening high temperature and decrease in appetite, and sometimes lymph node enlargement.[2] It is caused by bacillus, Mycobacterium Tuberculosis. Pulmonary Tuberculosis is the most contagious type, although it can affect other organ also, that is extra pulmonary TB. Though it is completely curable disease but if untreated leads to fatal consequences.[3] Tuberculosis bacteria spreads in air, probably after prolonged exposure to a patient having tuberculosis.[4] Globally, Tuberculosis is effecting population and millions of people developing TB disease. In 2019, world health organization reported, an estimated more than one million deaths due to deaths in Ten million effected people. This report also shows a bad situation among HIV-positive Millions of peoples are suffering from this fatal disease worldwide, especially in HIV positive cases and 208,000 TB related deaths among these HIV infected persons.[5] Tuberculosis prevalence is more in developing, low income countries as compare to developed countries. Nearly 70% of Tuberculosis cases are in Asia and Africa. The countries like India, china, Indonesia, Pakistan in Asia and South Africa, Nigeria in Africa, are mainly effected countries regarding Tuberculosis.[6] In this scenario, a slow progress in eliminating this fatal disease, make

1. MBBS MSPH, Additional District Health Officer,
2. MBBS, MSPH, Senior Lecturer, Prime University of Medical & Allied Health Sciences, Islamabad.
3. MBBS, M.Phil (Pharmacology), Associate Professor Pharmacology, Benazir Bhutto Medical College, Mirpur, AJK.
4. IPH, Assistant Professor, PUMHS.
5. MBBS, MSPH, Assistant Professor Community Health Sciences, PUMHSW.
6. MBBS, MSPH, Lecturer Public Health Department, Prime Institute of Health Sciences, Islamabad.
7. MBBS, M.Phil (Pharmacology), Assistant Professor, SMBBMU, Larkana.

Correspondence Address:
Dr. Masood Ahmed Unar
SMBBMU, Larkana.
fastmasood7@gmail.com

Article received on: 17/07/2024
Accepted for publication: 24/09/2024

an impression generally and especially in WHO ranks that we will not able to get the targets set in WHO strategy and world cannot end Tuberculosis disease, as a public health problem till 2035 as envisioned in the End TB Strategy.[7]

An urgent call for whole world regardless to their development, developed or developing, in a partnership, in September 2015, United Nation declared an Agenda for Sustainable Development for 2030.[8] This declaration contains 17 Sustainable Development Goals (SDGs), for all countries. In this regard, tuberculosis cannot to tackle without the help of each other. For above mentioned strategy, Diagnostic tools are key for tuberculosis management. For early diagnosis and treatment, GeneXpert MTB/RIF assay is regarded as one of the best available tool which helps in ending Tuberculosis.[9] This test provides the TB patients maximum benefits in this regard and give opportunity to interrupt the mycobacterium transmission which helps us to fulfil UN agenda. In high tuberculosis burden countries such as India, Pakistan, transmission of TB organism is main problem which can be solve by proper and early detection of organism and early and proper treatment. Initially, WHO recommended this test for MDR TB cases and HIV positive presumptive cases in 2010.[10] As this test was preferable and initial test for this category in 2010. After its established effectiveness, in 2013, keeping in mind its performance, WHO expanded its recommendations regarding the use of this new diagnostic tool that is GeneXpert MTB/RIF assay in the replacement of sputum smear microscopy.[11] GeneX-pert MTB/RIF assay become more popular as a diagnostic tool in Tuberculosis programs all over the world for detection of Mycobacterium. So, in most of the TB control setting this above mentioned test is used as the initial diagnostic test. This test also tells us about resistance of rifampicin, that's why, WHO recommended its use because of its ability to detect resistance to first line anti-Tuberculosis Drugs.[12]

We know that there is less sensitivity of conventional smear microscopy for detection of Mycobacterium tuberculosis in sputum specimen. In spite of this, sputum acid-fast smear remains in developing countries a main diagnostic tool, due to financial problem, for detection of Mycobacterium Tuberculosis in sputum of presumptive Tuberculosis cases. In Far East Asia country such as Thailand, a study aimed in 2013 to judge the diagnostic capability of GeneXpert by comparing with sputum smear microscopy which is used commonly. That study analyzed the sensitivity and specificity for Gene Xpert MTB/RIF assay and sputum smear microscopy were 95.3% and 86.4%, 60.5% and 98.5% respectively. This showed significantly higher sensitivity in GeneXpert and lower specificity in GeneXpert than sputum smear microscopy.[13] Other studies like study by Catherina C also concluded that GeneXpert test is more sensitive, accurate and effective at low cost for the early diagnosis of tuberculosis and especially when disease become resistance to most conventional drug therapies, early diagnosis may cure the progress to MDT TB.[14] This characteristic of the test make it preferable for use in Tuberculosis disease diagnostic facilities, especially its high sensitivity in smear- negative tuberculosis.[15]

METHODS

This study was a comparative cross-sectional study conducted in the outpatient department of District Head Quarter hospital and Taluka Head Quarter Hospitals of District Ghotki. The ethical approval was given by Ethical review committee of PUMHSW, Nawabshah with letter No: PUMHS/SBA/PVC/115, dated 30.6.2021.

The study population was Presumptive Tuberculosis cases in population of District Ghotki. This study included 350 patients by following the inclusion and criteria, Inclusion Criteria was, New Presumptive Pulmonary TB cases and relapse pulmonary TB cases, having their sputum, tested for GeneXpert assay and sputum smear microscopy. Exclusion Criteria was, Chronic chest diseases such as chronic obstructive pulmonary disease and extra pulmonary cases in which sputum smear microscopy cannot be done. The study design is comparative cross sectional, having duration of six months from October 2022 to April 2023, after the acceptance

of synopsis with a sampling technique is Non probability Convenience sampling. The inclusion and exclusion criteria was applied.

The diagnostic test performance of GeneXpert MTB/RIF assay was evaluated by comparing the results with sputum smear microscopy results and taking GeneXpert MTB/RIF as the reference investigation. A 2 x 2 contingency table was used for comparison. Frequencies were determined after the collection and analysis of data for true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated using the standard formulae. Data was analyzed using SPSS version 25, Data was expressed in number, percentage, and proportions in tables., all data from participant was collected after taking written consent, confidentiality of data was maintained.

RESULTS

In this comparative cross sectional study, sputum specimens of 350 patients were included. In Participants, 187 were males and 163 were females (Table-I), and according to age of patients see Table-II in this, 53 (15.2 %) patients having age are under 18 years, 152 (43.4%) patients having age between 19 and d 40 years, 103(29.4%) patients having age between 41 and 60 years and 42 (12%) patients are above 60 years. The mean age was 39 years (range: 13-98 years). The result of GeneXpert MTB/RIF test in detection of MTB DNA is detected in 61 (17.4%) samples (Table-III), whereas sputum smear microscopy test is positive in 35 (10%) samples. Among these 350 participants, GeneXpert MTB/RIF detected 61 cases, in which 26 (52%) were males and 24 (48%) were females (Table-IV). Data shows that GeneXpert MTB/RIF detection is approximately equally distributed in all age group. (See Table-V). And Table-VI shows the different measures of accuracy of tests.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	187	53.4	53.4	53.4
	Female	163	46.6	46.6	100.0
	Total	350	100.0	100.0	

Table-I. Sex group distribution of the participants (n=350)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Less than 18	53	15.2	15.2	15.2
	19-40	152	43.4	43.4	58.6
	41-60	103	29.4	29.4	88
	above 60	42	12	12	100
	Total	350	100.0	100.0	

Table-II. Age group distribution of the participants (n=350)

Serial No	Test performed	Positive result	Negative result	Total
1.	Gene Xpert MTB/RIF assay	61	289	350
2.	Sputum Smear microscopy	35	315	350

Table-III. Summary of sputum smear microscopy and GeneXpert test results

Serial No	Gender	Gene Xpert MTB/RIF Assay		Sputum Smear Microscopy	
		Detected	Non Detected	Positive	Negative
1	Male	32	146	23	160
2	Female	29	143	12	165
		61	289	35	315

Table-IV. Sex wise distribution of GeneXpert positive cases and sputum smear microscopy

Age Group	Participants	Genexpert Detected	Detection Percentage
Below 18 YRS	53	10	18.8
Between 19 to 40 YRS	152	27	17.7
Between 41 to 60 YRS	103	17	16.5
Above 60 YRS	42	07	16.6
Total	350	61	17.4

Table-V. Age group wise GeneXpert detection percentage

Sputum Smear Microscopy Results	Gene Xpert Results		
	Detected	Not Detected	Total
Positive	35 (TP)	0 (FP)	35
Negative	26 (FN)	289 (TN)	325
Total	61	289	350

Table-VI. Shows the measures of diagnostic accuracy of sputum smear microscopy

Measures of Diagnostic Accuracy of sputum Smear Microscopy

Sensitivity
True Positive / (True Positive + False Negative)
35/35+26 = 35/61 = 0.57 x 100 = 57

Specificity
True Negative / (True Negative + False Positive)
289/289 = 1x 100 =100

Positive-Predictive Value (PPV)
True Positive / (True Positive + False Positive)
35/35+0 = 1 x 100 =100

Negative-Predictive Value (NPV)
True Negative / (False Negative + True Negative)
289/315 = 0.917 x 100 = 91.7

Accuracy
True Positive + True Negative / (True Positive + False Negative + False Positive + True Negative)
324/350 = 0.925 x100 = 92.5

DISCUSSION

In recent past, Tuberculosis (TB) emerged as a global health problem with high prevalence. Timely diagnosis of this fatal disease is very much essential and important for initiating an effective management and it will then helpful for control of TB transmission in the community. As we are well aware that mycobacterial culture and sensitivity is the gold standard test, in this regard. But in past, mycobacterium Tuberculosis detection in sputum specimen in Pakistan and low and middle income countries mainly depends on conventional methods such as sputum smear microscopy. With the advancement in Diagnostic technologies, role of molecular diagnostic tools increases as of their good performance, in clinical sputum specimens and others within limited time. As we know, variation ranging 20% to 80% of sputum smear in detection of mycobacterium, making this diagnostic tool less reliable.[16] In contract, GeneXpert MTB/RIF assay reliability is much better incomparable to sputum smear microscopy. The aim of this study is to evaluate the performance of GeneXpert MTB/RIF when comparing with sputum smear microscopy.

This study included different age groups are approximately equally effected, but males are more effected than females. Global TB report of 2019 also showed male predominance in presumptive Tuberculosis cases in Pakistan.[17] Low positive cases in female as compare to males is due to unawareness about sputum collection in females, this was a analysis of positive cases among both sex. It is also noticed cases of Tuberculosis are less in Adolescents and children as per their population size. This may be because wide spread use of BCG vaccine in Expanded program of immunization in Pakistan. BCG vaccination coverage in Pakistan is much better as compared to other vaccines that is 80 %, which is good explanation for the decrease TB prevalence as per population size in adolescents and children in our study.[18]

This study reveals that 57 % sensitivity for sputum smear microscopy, which is moderate. But this diagnostic tool showed high specificity, positive-predictive value, and negative-predictive value taking the sputum GeneXpert MTB/RIF assay as the reference test. In this study, 26 confirmed Tuberculosis cases were missed by Sputum smear microscopy. GeneXpert MTB/RIF test which detected 61 cases in our study. In some other studies, similar findings were observed. The reason behind these missed cases or the lower sensitivity of sputum smear microscopy is because of it needs 5,000 bacilli/ml of sputum specimen to detect mycobacterium properly and for GeneXpert MTB/RIF assay is 136 bacilli/ml is needed to detect mycobacterium properly in aspecimen.[19] This is the main reason in developing countries, MDR

Tuberculosis is increasing day by day, because

of lack of accurate or advanced diagnostic tools. The main theme for introduction of reliable and advanced diagnostic tool is to tackle missed cases of tuberculosis which prolongs morbidity and ultimate increase in morbidity in the affected individual.

The infectious rate of smear negative cases are less in comparison of smear positive cases , but transmission and spread of Tuberculosis also depend upon these missed cases, as precautions are not taken because of unawareness of cases. As documented, tuberculosis transmission percentage is 10%-20%, in smear-negative cases of pulmonary Tuberculosis.[20] If we minutely go through the entire period of patient from being infected, diseased, diagnosed and treated. We came to know that main problem of controlling this disease is timely and proper diagnosis. So, during this period, Patient, his family and his community suffer financial as well as mental effects of disease. Therefore, this study as well as other studies are evident that GeneXpert is advance and more sensitive and specific test for the diagnosis of Pulmonary Tuberculosis. It is also rapid and less expensive to culture and sensitive method. So, in the interest of Patient, GeneXpert testing where available, will be beneficial for patient in terms of reducing morbidity in the patients and other direct as well as indirect costs of Tuberculosis disease due to missed cases in presumptive Tuberculosis especially in smear-negative pulmonary Tuberculosis.

CONCLUSION

This Study concluded that, the GeneXpert MTB/RIF Assay outperforms conventional diagnostic tools like sputum smear microscopy in diagnostic accuracy and performance. It is particularly effective in smear negative microscopy results, particularly in low mycobacterium loaded sputum. The rapid and simple test makes it a valuable tool for rapid diagnosis of Tuberculosis.

RECOMMENDATIONS

Keeping in view, the findings of our study. I recommend the policy makers to take necessary steps to address following

- Availability of GeneXpert MTB/RIF assay should be made where detection rates are low due to dependency on only sputum smear microscopy.
- Staff working in GeneXpert laboratories should be trained well and they should be free from other hospital responsibility, so that they can work properly.
- GeneXpert also detects RR that is rifampicin resistance. I recommend that previously treated cases should be tested for GeneXpert MTB/RIF assay to detect timely cases of MDR.
- I also recommend that more research is needed to find out more ways by introducing better and advance diagnostic technology which will improve the present situation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SOURCE OF FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Cave AJ, Demonstrator A. **The evidence for the incidence of tuberculosis in ancient Egypt.** Br J Tuberc. 1939 Jul 1; 33(3):142-52.

2. Farina E, D'Amore C, Lancella L, Boccuzzi E, Ciofi Degli Atti ML, Reale A, Rossi P, Villani A, Raponi M, Raucci U. **Alert sign and symptoms for the early diagnosis of pulmonary tuberculosis: Analysis of patients followed by a tertiary pediatric hospital.** Ital J Pediatr. 2022; 48(1):90. https://doi.org/10.1186/s13052-022-01288-5

3. Khan MA, Bilal W, Asim H, Rahmat ZS, Essar MY, Ahmad S. **MDR-TB in Pakistan: Challenges, efforts, and recommendations.** Ann Med Surg. 2022; 79:104009. https://doi.org/10.1016/j.amsu.2022.104009

4. Das DK, Khajanchi S, Kar TK. **Influence of multiple re-infections in tuberculosis transmission dynamics: A mathematical approach.** In: 2019 8th Int Conf Model Simul Appl Optim (ICMSAO); 2019 Apr 15. IEEE. pp. 1-5.

5. Migliori GB, Garcia-Basteiro AL. **Predicting the effect of improved socioeconomic health determinants on the tuberculosis epidemic.** Lancet Glob Health. 2018 May 1; 6(5):e475-6.

6. Chakaya J, Khan M, Ntoumi F, Aklillu E, Fatima R, Mwaba P, Kapata N, Mfinanga S, Hasnain SE, Katoto PD, Bulabula AN. **Global Tuberculosis Report 2020–Reflections on the Global TB burden, treatment and prevention efforts.** Int J Infect Dis. 2021 Dec 1; 113:S7-12.

7. John CA. **Realizing the World Health Organization's End TB Strategy (2016-2035): How can social approaches to tuberculosis elimination contribute to progress in Asia and the pacific?** Trop Med Infect Dis. 2019; 4(1):28. https://doi.org/10.3390/tropicalmed4010028

8. Floyd K, Glaziou P, Houben RMGJ, Sumner T, White RG, Raviglione M. **Global tuberculosis targets and milestones set for 2016-2035: Definition and rationale.** Int J Tuberc Lung Dis. 2018; 22(7):723-730. https://doi.org/10.5588/ijtld.17.0835

9. Getahun DA, Layland LE, Hoerauf A, Wondale B. **Impact of the use of GeneXpert on TB diagnosis and anti-TB treatment outcome at health facilities in Addis Ababa, Ethiopia in the post-millennium development years.** PLoS One. 2023; 18(8):e0289917.

10. Brown S, Leavy JE, Jancey J. **Implementation of GeneXpert for TB Testing in Low- and Middle-Income Countries: A systematic review.** Global Health Sci Pract. 2021; 9(3):698-710. https://doi.org/10.9745/GHSP-D-21-00121

11. Sorsa A, Kaso M. **Diagnostic performance of GeneXpert in tuberculosis-HIV co-infected patients at Asella Teaching and Referral Hospital, Southeastern Ethiopia: A cross-sectional study.** PLoS One. 2021; 16(1):e0242205. https://doi.org/10.1371/journal.pone.0242205

12. Horne DJ, Kohli M, Zifodya JS, Schiller I, Dendukuri N, Tollefson D, et al. **Xpert MTB/RIF and Xpert MTB/RIF Ultra for pulmonary tuberculosis and rifampicin resistance in adults.** Cochrane Database Syst Rev. 2019; 6(6):CD009593.

13. Pinyopornpanish K, Chaiwarith R, Pantip C, Keawvichit R, Wongworapat K, Khamnoi P, et al. **Comparison of Xpert MTB/RIF assay and the conventional sputum microscopy in detecting Mycobacterium tuberculosis in Northern Thailand.** Tuberc Res Treat. 2015 Apr 30; 2015.571782.

14. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, Tahirli R, et al. **Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: A multicentre implementation study.** Lancet. 2011; 377(9776):1495-1505.

15. Cuong NK, Ngoc NB, Hoa NB, Dat VQ, Nhung NV. **GeneXpert on patients with human immunodeficiency virus and smear-negative pulmonary tuberculosis.** PLoS One. 2021; 16(7):e0253961.

16. Dzodanu EG, Afrifa J, Acheampong DO, Dadzie I. **Diagnostic yield of fluorescence and Ziehl-Neelsen staining techniques in the diagnosis of pulmonary tuberculosis: A comparative study in a District Health Facility.** Tuberc Res Treat. 2019 Apr 10; 2019.

17. Qadeer E, Fatima R, Yaqoob A, Tahseen S, Ul Haq M, Ghafoor A, et al. **Population-based national tuberculosis prevalence survey among adults (>15 years) in Pakistan, 2010-2011.** PLoS One. 2016 Feb 10; 11(2):e0148293.

18. Butt M, Mohammed R, Butt E, Butt S, Xiang J. **Why have immunization efforts in Pakistan failed to achieve global standards of vaccination uptake and infectious disease control?** Risk Manag Healthc Policy. 2020; 13:111.

19. Umair M, Siddiqui SA, Farooq MA. **Diagnostic accuracy of sputum microscopy in comparison with GENEXPERT in pulmonary tuberculosis.** Cureus. 2020 Nov 8; 12(11):e11383.

20. Sinshaw W, Kebede A, Bitew A, Tesfaye E, Tadesse M, Mehamed Z, et al. **Prevalence of tuberculosis, multidrug-resistant tuberculosis and associated risk factors among smear-negative presumptive pulmonary tuberculosis patients in Addis Ababa, Ethiopia.** BMC Infect Dis. 2019 Dec; 19(1):1-5.

AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Rao Aftab Ahmed	Main author and researcher of the study.	
2	Seema Qazi	Assisted in search of literature review.	
3	Uzma Riaz	Writing and final drafting of article.	
4	Miss Faiqa Memon	Data collection and analysis.	
5	Hanna Khair Tunio	Assisted in synopsis preparation.	
6	Sana Aqeel	Helped in analysis.	
7	Masood Ahmed Unar	Preparation of article and corresponding author.	