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Role of Culture Free Rapid ImmunoAssay (ELISA) and Genexpert (MTB/RIF) Technology in the Diagnosis of Tuberculosis

[1]Shahida Hussain, [1]Sikander Sultan, [1]Saba Riaz, [2]Hajra Hussain, [3]Chetan Lal

ABSTRACT

Introduction: Prolonged laboratory processes are major hurdles in the early diagnosis of tuberculosis. Prompt management of this disease is utmost necessary to control its spread. For this purpose, use of rapid and reliable laboratory testing methods for the diagnosis of *Mycobacterium tuberculosis* are crucial alongwith timely infection management and preventing its transmission to the close contact population.

Aims and Objective: The aim of this study was to establish a diagnostic-connection between culture free cost effective rapid immuno-assay (IgG and IgM) and GeneXpert (MTB/RIF) technology for the detection of *Mycobacterium tuberculosis* within the shortest possible turnaround time.

Place and Duration of Study: This cross-sectional study was carried out at Institute of Microbiology and Molecular Genetic, University of the Punjab, Pakistan in collaboration with the Department of Pulmonology and TB Outpatient Department (OPD) at Jinnah Hospital, Lahore over the time of one year (2019-2020).

Material and Methods: To achieve the aim, a total of 180 (pulmonary and extra-pulmonary) samples along with blood samples were collected from clinical suspects of Tuberculosis. All collected samples were subjected to AFB microscopy, GeneXpert (MTB/RIF) assay and Enzyme-linked Immunosorbent Assay (ELISA) for IgG and IgM. The diagnostic sensitivity and specificity of both methods were determined by using the solid culture as the gold standard.Study data collected through questionnaire was entered and analyzed using Statistical Package for Social Sciences (SPSS version 20).p value of ≤ 0.05 was considered significant.

Results: The overall sensitivity and specificity of GeneXpert (MTB/RIF) assay were 98.2 % and 82.4% respectively, whereas, for IgG (ELISA) antibodies, it was 50.0% and 76.4% and for IgM (ELISA) antibodies 50.4% and 97.1% respectively.

Conclusion: It was concluded that with fully automated GeneXpert (MTB/RIF) technology, maximum sensitivity and specificity can be achieved for diagnosis of tuberculosis within 2 hours.

Keywords: GeneXpert (MTB/RIF), Mycobacterium Tuberculosis, IgG, IgM

INTRODUCTION

Tubercle-bacilli or Tuberculosis (TB) is a lethal infectious disease, caused by single infectious agent "*Mycobacterium tuberculosis*" (MTB), which is more prevalent in developing countries, including Pakistan[1]. In the human population TB remains a life-threatening disease after HIV/AIDS. Despite the fact that, TB is curable and preventable, but according to published data, one-third world population is infected from TB[2]. TB is somehow disease of poverty because it mainly effects human population with low socio-economic status[3]. It is a transmittable airborne infection that is spread by coughing, spitting and sneezing of contagious patients[4]. Population of around 25,000 per day showed evidence of development of active TB and more than 4,500 people died due to TB, which emphasizes the demand for the rapid diagnostic tools and effective intervention approaches[5]. Currently, different microbiological (direct staining and culture methods), immunological (ELISA for IgG, IgM) and molecular (Real-time Polymerase Chain Reaction-PCR) approaches are available for the diagnosis of TB. All these available diagnostic tools have different diagnostic sensitivity and specificity against *MTB*. Although culturing of *MTB* on different selective medium (Solid or Liquid) is considered as the definitive method or gold standard

[1]*Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore. Pakistan.*
[2]*Deparment of Gynae & Obs, District Headquarter Hospital, Gilgit Baltistan, Pakistan.*
[3]*Department of Microbiology, Federal Postgraduate Medical Institute, Shaikh Zayed Hospital, Lahore, Pakistan.*

Correspondence:
Dr. Chetan Lal, *Assistant Professor, Department of Microbiology, Federal Postgraduate Medical Institute, Shaikh Zayed Hospital, Lahore, Pakistan*
Email: drchetanlal@hotmail.com

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for TB. Currently, conventional microbiological diagnostic method such as microscopy of sputum smear or growing the *MTB* on solid or liquid culture are used as definitive diagnostic tool for the identification of active TB[6]. These methods still have diagnostic challenges due to some drawbacks. Smear microscopy is affordable method but has proven low sensitivity and specificity and solid culture is laborious and needs sophisticated technical facilities[7]. Furthermore, molecular amplification methods are being more promising but still unaffordable by high burden TB communities, as these assays require very dedicated and expensive laboratory setups. Therefore, prompt and accurate diagnosis of TB is still crucial for developing countries to fight against the global TB epidemic.

However, demand for more alternative diagnostic methods have been under process since long time. Availability of rapid, cheap and point of care tests at all health care levels of community are critically needed to halt the TB spread by rapid diagnosis. It will result in effective on time treatment or monitoring of disease management. The endorsement of real time PCR based GeneXpert MTB/RIF (Cepheid Inc., CA, USA) technology by WHO in developing countries for rapid diagnosis along with detection of drug (Rifampicin) resistance in just 2 hours is encouraging[8,9]. The serological based diagnostic approach by measuring the presence of IgG and IgM antibodies against specific MTB antigen could be proven to be a useful companion to microscopic examination for sputum of patients for the screening of latent or active TB cases[10]**.** These tests are rapid, cost effective and can be used as point of care test in resource limited diagnostic settings. The efficacy of these kind of sero-diagnostics for the detection of antibodies in the TB patients have been widely assessed[11].It is extremely important to halt the TB transmission by detecting it at an early stage. To diagnose, monitor and assess the treatment outcomes, host biomarkers play a very vital role. These immunological markers potentially predict the culture success or failure. Therefore, reduced time for differential diagnosis and initiation of treatment. Thus, the focus of present study was to determine the sensitivity, specificity and other statistical parameters of molecular GeneXpert MTB/RIF Assay and compare the immunological profile (IgG and IgM) by ELISA with negative and positive solid cultures, as well as smear microscopy to identify possibly useful differences as an aid in diagnosis.

MATERIAL AND METHODS

Study Setting and Study Design:

This cross-sectional study was conducted in the Institute of Microbiology and Molecular Genetics, in collaboration with the Department of Pulmonology and TB Outpatient Department (OPD) at Jinnah Hospital, Lahore (JHL) and Citi Laboratory and Research Center, LahoreThis study was approved by the Ethical Review Board of Punjab University vide number 9043 dated 14th Nov 2019.One hundred and eighty (range 10 to 99 years) TB suspects were selected based on clinical symptoms and radiological evidence over the time period of 1 year (2019-2020). The complete patient history such as demographic profiles including age, sex, occupation, socioeconomic status, address, contact number, relevant history regarding the intensity and duration of the symptoms were recorded on the Performa after taking consent from patients. The number of patients were rigorously selected to include in study. Patients on immune-suppressive drugs, HIV positive cases and those receiving Anti-tuberculosis drugs were excluded. Moreover, hemolyzed blood samples or insufficient sputum and extra-pulmonary samples were also excluded.

Sample Collection and Processing:

PTB and EPTB samples were divided into 3 parts, one for AFB smear, 2nd for solid culture and 3rd for the WHO endorsed GeneXpert (MTB/RIF) assay, along with 3cc blood in clotted vials for the determination of IgG and IgM level by ELISA methods were sent to TB laboratory of Pathology Department of Allama Iqbal Medical College, Lahore (Fig-1) The EPTB-samples were concentrated by using cyto-centrifugation machine and resultant deposit was furthers processed for the Acid-fast smear, GeneXpert (MTB/RIF) and solid culture as per WHO recommendation[12].

Acid Fast Smear and Bacilli Culture:

All clinical specimens were subjected to acid fast smear by Ziehl-Neelsen (ZN) staining for the examination of presence of acid-fast bacilli (AFB) under microscope as per WHO protocol[13]. For bacilli culture, a total of 200 µL of a decontaminated samples were used to grow the bacilli in solid culture or Löwenstein-Jensen culture medium as per WHO standard protocol in a Biosecurity Level III laboratory [12].

GeneXpert (MTB/RIF) Assay:

For the nucleic acid detection of mycobacterium tuberculosis DNA, all samples were directly subjected to GeneXpert (MTB/RIF) assay as per protocols given by manufacturer[14].

Immunoglobin IgM and IgG Detection by ELISA:

The determination of specific immunoglobin (IgM and IgG) in serum of suspected patients were performed by standard ELISA method by using commercially available ELISA kit (Genix Diagnostics GmbH, Germany)[15].

Statistical Data Analysis:

The interpretation of study data collected through questionnaire was analyzed descriptively and analytically by using Statistical Package for Social Sciences (SPSS version 20). Diagnostic validity in terms of sensitivity and specificity of AFB smear, GeneXpert (MTB/RIF) and ELISA were calculated by using LJ culture as gold standard method for the diagnosis of tuberculosis.

RESULTS

In this study, a total of 180 samples from different suspected TB patients were included. The cohort was comprised of pulmonary (n=120) and extra pulmonary (n=60) TB suspects. The overall mean age of study participants was 49.6±4.7 years. However, 108 (60%) of them were male and 72 (40%) were females. The results of present study, shows that, TB is more common in men as compared to females (Table-1). This correlation of TB-Males is due to different risk factors likes smoking, nature or occupation and outdoor activities of males in our society. Incidence of TB at different ages, in the given population was studied and showed that amongst the total number of patients, 98 had age of < 30 years, whereas 40 patients were above 50 years. It was observed that people belonging to lower income class groups have more chances of developing TB. The percentage of different socio-economic groups in the given population suffering from the disease were 78.9% in lower class, 16.7% in middle class and 4.4 % in higher class (Table-1).

Of the 180 specimens, 120 (66.6 %) were pulmonary and 60 (33.3%) were EP. Among all diagnostic tools used in this study, GeneXpert (MTB/RIF) assay 122 (67.7%) showed the highest detection rate for MTB, followed by LJ culture 112 (62.1%), IgG (ELISA) 78 (41.3%) and ZN smear 116 (64.4%) respectively. IgM (ELISA) 60 (33.3%) showed the lowest detection rate for MTB as compared to other diagnostic tools (Fig-2). The sensitivity and specificity of GeneXpert (MTB/RIF) were also calculated by comparing the results of GeneXpert (MTB/RIF) with gold standard (LJ culture). The sensitivity and specificity of GeneXpert (MTB/RIF) was 98.2% and 82.4% respectively and the difference was statistically significant (P <0.05) (Table-2).The performance of the ZN, LJ culture, GeneXpert and ELISA in terms of time of diagnosis were also compared in our study. The results showed that the GeneXpert (MTB/RIF) and ELISA methods were more rapid with 2- 3 hours of mean time of positivity as compared to conventional techniques (Table-3). In current study GeneXpert (MTB/RIF) shows high sensitivity and specificity as compared to other methods (Table-2). Our results and finding agreed with the WHO (2010) recommendation of using GeneXpert (MTB/RIF) for rapid detection of TB in both smear negative and smear positive patients. WHO policy updated in 2014, recommended the implementation of GeneXpert (MTB/RIF) assay in the diagnosis of EPTB cases and smear negative cases (Table-3).

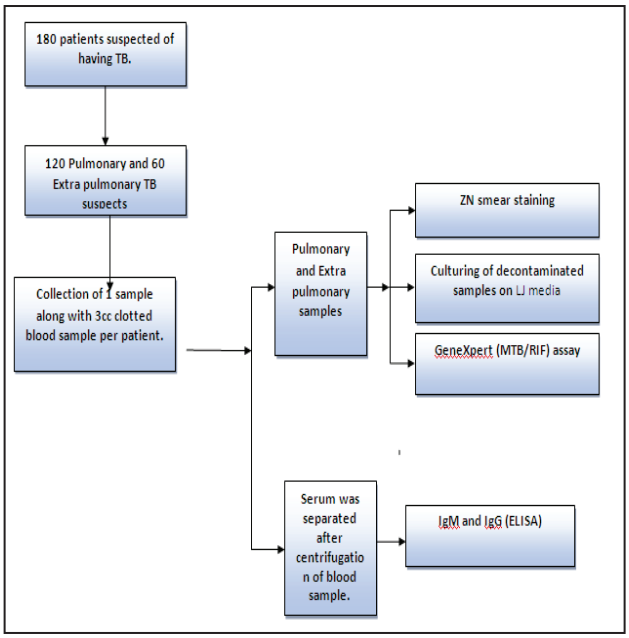

Fig-1: The Flow Chart Shows the Sample Collection and Processing of TB Suspects.

Parameters	Groups	Frequency	Percent (%)
Age	10 – 30 yrs	98	54.0
	31 - 50 yrs	52	28.0
	51 - 70 yrs	18	10.0
	71 - 90 yrs	12	7.6
Gender	Male	108	60.0
	Female	72	40.0
Socio-Economic Status	Lower class	142	78.9
	Middle class	30	16.7
	Upper class	8	4.4

Table-1: Characteristics Of Demographic Groups of Study Population (N=180)

ype Culture as Gold Standard	ZN smear Microscopy		GeneXpert (MTB/RIF)		IgM (ELISA)		IgG (ELISA)	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Pulmonary n=120	56/86	30/34	84/86	24/34	38/86	32/34	42/86	26/34
	67.4%	88.1%	97.6%	70.5%	44.0%	94.4 %	49.0%	76.1%
Extra pulmonary n=60	2/26	26/26	26/26	32/34	18/26	34/34	14/26	26/34
	7.7%	100 %	100%	94.6%	69.2%	100 %	58.8%	76.5%
Total n=180	60/112	32/56	10/112	56/68	56/112	66/68	56/112	52/68
	53.6%	94.1%	98.2%	82.4%	50.0%	97.1 %	50.0%	76.5%

Table-2: Sensitivity and specificity of different diagnostic test by comparing with gold stand method (LJ culture).

Table-2 shows the sensitivities and specificities of ZN smear microscopy, Gene-Xpert (MTB/RIF) and ELISA (IgM and IgG) test with respect to different specimen groups upon comparison with culture results. The results showed that Gene-Xpert (MTB/RIF) assay showed high detection rate in both groups (pulmonary and EP) as compared to other methods used in this study with sensitivity and specificity of 98.2% and 82.4% respectively.

Test	Time of Positivity	Mean Time of Positivity	Time of Negativity
Gene-Xpert (MTB/RIF) assay	2 Hours	2 Hours	2 Hours
LJ Culture	3-6 Weeks	5 Weeks	8 Weeks
ELISA	3-4 Hours	4 Hours	4 Hours
ZN Smear Microscopy	1 Hour	1 Hour	1-24 Hour

Table-3: Comparison Of ZN Smear, LJ Culture, ELISA And Gene-Xpert (MTB/RIF) Assay in Term of their time Of Diagnosis.

Table-3 shows that Gene-Xpert (MTB/RIF) has proved to be most rapid diagnostic tool for the diagnosis of TB patients as compared to other conventional microbiological tools.

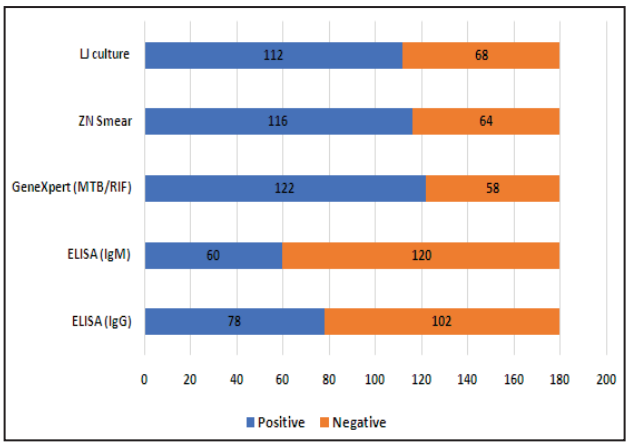

Fig-2: Diagnostic Efficacy of Different Diagnostic Tool Used In The Study.

DISCUSSION

Tuberculosis (TB) had been a main public health issue globally and being the largest killer of adult deaths in the developing countries (like Pakistan) from a single infectious agent (*Myco-bacterium tuberculosis)*[16]. Time factor is matter of great importance in the diagnosis of TB. The conventional methods presently used for isolation, identification and antibiotic sensitivity of mycobacterium are either costly or time consuming. That's why, a definitive diagnosis of mycobacterium infection depends on rapid identification of the *MTB* in clinical samples. Therefore, the key objective of the current study was to evaluate the new emerging techniques like GeneXpert (MTB/RIF) and ELISA and their comparison with conventional techniques (LJ culture and AFB staining) for the diagnosis of tuberculosis in local population. This study also investigated different techniques used to detect mycobacterium, finding the relationship between them and their validity on using LJ culture as a gold standard for diagnosis and the relative comparison of their sensitivity. As ZN staining is affordable, convenient and easy to perform for routine diagnosis of TB but it provides the preliminary diagnosis and it is not considered as an accurate method because it can't differentiate between viable bacteria and dead one[17]. Although the sensitivity of this method, with reference to LJ culture, is 25-75% and specificity is 95%[18]. In our study, prevalence of TB and its relationship with demographic characteristic; age, gender and socio-economic status have been investigated. The result of our study indicates that young people (> 30 years) are at higher risk of TB infection than old age (54%) (Table-2). The reason behind this is due to direct contact with bacillary load carry on daily life expenses and nature of disease. In our study the

prevalence of TB in the older age range seems to be less frequent, which may be due to problems facing in attending the TB centers or hospitals for old age group. These results have also been agreed with previous results[19,20]. A gender difference in TB has been an interesting topic of research for many years. Amongst males 60% of the population and 40% females have been documented for this disease in the given population (Table-1). This result agrees with other studies[21,22]. TB is also known as a disease of poverty because of the lack of proper diagnostic tools, and treatment at affordable rates in developing countries. Many of the infected people cannot manage to pay for TB drugs. In current study the most of suspected population belonged to low scio-economic group (142/180), followed by middle class (30/180) and upper class (8/180) (Table-1). The reason behind this might be due to overcrowded living conditions, malnutrition, lack of hygienic environment/food, non-availability of proper management programs in government sectors.

ELISA based TB serology is a very useful complementary tool in the diagnosis and monitoring of mycobacterial infections[23]. IgM and IgG antibodies are developed in the body against *MTB,* and they can be detected by ELISA in sera of patient[24]. In our study, the sensitivity and specificity of IgM (antibodies) was 50 %, 97.1% respectively and in case of IgG the sensitivity and specificity was found to be 50% and 76.5% respectively (Table-2). The sensitivities of IgM and IgG were comparatively the same as the results observed by Bam et al. (2009), which were 48.0% and 76.6%[24]. Our results are in favor of updated WHO policy (2014), in which WHO strongly ban TB sero-diagnosis, because of these false negative results of ELISA[25]. The reason behind this failure may be due to the use of a single antigen-based ELISA assay. We evaluated the diagnostic sensitivity and specificity of GeneXpert both for PTB and EPTB. These results of current study have proved that GeneXpert (MTB/RIF) assay is a better method for diagnosis of TB because of its close comparability to the LJ culture. When we compared the results of GeneXpert (MTB/RIF) with LJ culture, the sensitivity and specificity of GeneXpert (MTB/RIF) in pulmonary samples was found to be 97.6% and 70.5% respectively (Table-2). Our results are consistent with those reported by several other workers[26,27]. Comparable efficacy was observed in extra-pulmonary samples showing sensitivity (100%) and specificity (94.6%) by GeneXpert (MTB/RIF) assay (Table-2). It has also been observed that GeneXpert (MTB/RIF) could detect 25% more positive cases as compared to ZN microscopy, indicating 2-3 times more sensitivity of GeneXpert for EPTB (Table-2). In agreement, Panayotis et al., in their study on EPTB samples reported a sensitivity and specificity of 100% and 91.6% again indicating a higher sensitivity of GeneXpert as compared to other methods[28]. These results are like previous studies, which have reported test sensitivity ranging from 57 to 83% in cases of culture positive but smear negative TB and 98 to 100% in cases of culture and smear positive cases, while the test specificity remained at 99% to 100%[29].

In the diagnosis of tuberculosis time factor is of crucial importance which helps in starting correct and result oriented therapy. In GeneXpert the time for detection of tuberculosis is 0 days, and results become available in less than two hours, as compared with 1 day (0–1) for microscopy, 30 days (23-43) for LJ culture, and 16 days (13-21) for liquid culture[30]. It is, therefore, concluded that GeneXpert MTB/RIF assay has become a more accurate and rapid method for diagnosis of PTB and EPTB as compared to LJ culture in both smear negative (97%) and positive samples (96.8%). In the current study we observed 98.7% sensitivity and 82.3% specificity for all cases of TB (Pulmonary and EP). GeneXpert test has rapid turnover with less biohazards, less prone to cross-contamination, only minimal training required, excellent performance, easy to use, fully automated and has a shorter detection time, make this technique a very attractive tool for diagnosis of *MTB* from clinically suspected samples, which are all important considerations in while selecting a diagnostic tool for health care system.

CONCLUSION

In the current study, we observed 98.7% sensitivity and 82.3% specificity for all cases of TB (Pulmonary and EP). GeneXpert test has rapid turnover with less biohazards, less prone to cross-contamination, minimal training required, excellent performance, easy to use, fully automated and has a shorter detection time, make this technique a very attractive tool for diagnosis of *MTB* from clinically suspected samples, which are all important considerations while selecting a diagnostic tool for health care systems.

REFERENCES

1. Natarajan A, Beena P, Devnikar AV, Mali SJIJoT. A systemic review on tuberculosis. 2020;67(3):295-311.
2. Organization WH. WHO consolidated guidelines on drug-resistant tuberculosis treatment: World Health Organization; 2019.
3. Das D, Das S, Pandey M, Bhattacharyay DJEJoMP. In silico analysis of phytochemicals from Mucuna pruriens (L.) DC against Mycobacterium tuberculosis causing tuberculosis. 2020;31(4):19-24.
4. Koupaei M, Naimi A, Moafi N, Mohammadi P, Tabatabaei FS, Ghazizadeh S, et al. Clinical characteristics, diagnosis, treatment, and mortality rate of TB/COVID-19 coinfectetd patients: a systematic review. 2021;8:2188.
5. Guler SA, Bozkus F, Inci MF, Kokoglu OF, Ucmak H, Ozden S, et al. Evaluation of pulmonary and extrapulmonary tuberculosis in immunocompetent adults: a retrospective case series analysis. 2015;24(1):75-9.
6. Quradha MM, Khan R, Rehman M-u-, Abohajeb AJNpr. Chemical composition and in vitro anticancer, antimicrobial and antioxidant activities of essential oil and methanol extract from Rumex nervosus. 2019;33(17):2554-9.
7. Purohit M, Mustafa TJJoc, JCDR dr. Laboratory diagnosis of extra-pulmonary tuberculosis (EPTB) in resource-constrained setting: state of the art, challenges and the need. 2015;9(4):EE01.
8. 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. 2019(6).
9. Kohli M, Schiller I, Dendukuri N, Yao M, Dheda K, Denkinger CM, et al. Xpert MTB/RIF Ultra and Xpert MTB/RIF assays for extrapulmonary tuberculosis and rifampicin resistance in adults. 2021(1).
10. Little KM, Pai M, Dowdy DWJPO. Costs and consequences of using interferon-γ release assays for the diagnosis of active tuberculosis in India. 2015;10(4):e0124525.
11. Baumann R, Kaempfer S, Chegou NN, Oehlmann W, Spallek R, Loxton AG, et al. A subgroup of latently Mycobacterium tuberculosis infected individuals is characterized by consistently elevated IgA responses to several mycobacterial antigens. 2015;2015.
12. Chihota V, Grant A, Fielding K, Ndibongo B, Van Zyl A, Muirhead D, et al. Liquid vs. solid culture for tuberculosis: performance and cost in a resource-constrained setting. 2010;14(8):1024-31.
13. Iram S, Zeenat A, Hussain S, Yusuf NW, Aslam MJPjoms. Rapid diagnosis of tuberculosis using Xpert MTB/RIF assay-Report from a developing country. 2015;31(1):105.
14. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. 2010;363(11):1005-15.
15. Escobar-Gutierrez A, Amezcua-Chavarria ME, Pasten-Sanchez S, Ramirez-Casanova E, Cazares J, Granados G, et al. Enzyme-linked immunosorbent assay (ELISA) with mycobacterial crude antigens for the sero-epidemiological diagnosis of active tuberculosis. 1996;64(4):417-27.
16. Wani AA, Kait RJSJMPS. Tuberculosis (TB) Silent Killer, Challenges and Stategies to Manage Tuberculosis during COVID-19 Pandemic: A Review. 2021;7(11):571-4.
17. Laifangbam S, Singh HL, Singh N, Devi K, Singh NJKUmj. A comparative study of fluorescent microscopy with Ziehl-Neelsen staining and culture for the diagnosis of pulmonary tuberculosis. 2009;7(27):226-30.
18. Stover CK, Warrener P, VanDevanter DR, Sherman DR, Arain TM, Langhorne MH, et al. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. 2000;405(6789):962-6.
19. Li H, Javid BJNRI. Antibodies and tuberculosis: finally coming of age? 2018;18(9):591-6.
20. Bae W, Park KU, Song EY, Kim SJ, Lee YJ, Park JS, et al. Comparison of the sensitivity of QuantiFERON-TB Gold In-Tube and T-SPOT. TB according to patient age. 2016;11(6):e0156917.
21. Weiss M, Somma D, Karim F, Abouihia A, Auer C, Kemp J, et al. Cultural epidemiology of TB with reference to gender in Bangladesh, India and Malawi [Special section on gender and TB]. 2008;12(7):837-47.
22. Allotey P, Gyapong MJTIJoT, Disease L. Gender in tuberculosis research [Special section on gender and TB]. 2008;12(7):831-6.
23. Richomme C, Boadella M, Courcoul A, Durand B, Drapeau A, Corde Y, et al. Exposure of wild boar to Mycobacterium tuberculosis complex in France since 2000 is consistent with the distribution of bovine tuberculosis outbreaks in cattle. 2013;8(10):e77842.
24. Bam N, Karn RJJoIoMN. IgM and IgG antibodies in tuberculosis. 2009;31(3):34-40.
25. Organization WH. Drug-resistant TB: surveillance and response: supplement to global tuberculosis report 2014. World Health Organization; 2014.
26. Alvarez-Uria G, Azcona JM, Midde M, Naik PK, Reddy S, Reddy RJTr, et al. Rapid diagnosis of pulmonary and extrapulmonary tuberculosis in HIV-infected patients. Comparison of LED fluorescent microscopy and the geneXpert MTB/RIF assay in a district hospital in India. 2012;2012.
27. Chang K, Lu W, Wang J, Zhang K, Jia S, Li F, et al. Rapid and effective diagnosis of tuberculosis and rifampicin resistance with Xpert MTB/RIF assay: a meta-analysis. 2012;64(6):580-8.
28. Ioannidis P, Papaventsis D, Karabela S, Nikolaou S, Panagi M, Raftopoulou E, et al. Cepheid GeneXpert

MTB/RIF assay for Mycobacterium tuberculosis detection and rifampin resistance identification in patients with substantial clinical indications of tuberculosis and smear-negative microscopy results. 2011;49(8):3068-70.

29. Karigoudar rm, karigoudar mh, wavare sm, kakhandki l, bagali sjijop, sciences p. Use of cartridge based nucleic acid amplification test for rapid detection of mycobacterium tuberculosis in pulmonary and extrapulmonary tuberculosis. 2021;13(2):29-33.

30. 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. 2011;377(9776):1495-505.

The Authors:

Shahida Hussain,
PhD Scholar,
Department of Microbiology and Molecular Genetics,
University of the Punjab, Lahore.

Dr. Sikander Sultan,
Professor of Microbiology,
Department of Microbiology and Molecular Genetics,
University of the Punjab, Lahore.

Dr. Saba Riaz,
Associate Professor of Microbiology,
Department of Microbiology and Molecular Genetics,
University of the Punjab, Lahore

Dr. Hajra Hussain,
Women Medical Officer,
Department of Gynae & Obstatrics,
District Headquarter Hospital, Gilgit Baltistan.

Dr. Chetan Lal,
Assistant Professor,
Department of Microbiology, Federal Postgraduate Medical Institute,
Shaikh Zayed Hospital, Lahore.

Authorship:

SH: Data collection, Bench work, Data analysis
SS: Research Conceptualization, Supervisor
SR: Co-Supervisor
HH: Manuscript Drafting
CL: Manuscript Editing