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Comparison of Ziehl Neelsen Microscopy with GeneXpert for Detection of MycobacteriumTuberculosis

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Abstract:
Background: *Tuberculosis (TB) is a transmissible bacteriological ailment triggered by Mycobacterium tuberculosis (MTB) and is still one of the biggest challenges for developing countries.Main reasons for using the ZN smear microscopy is its low cost, high specificity, does not require sophisticated equipment or high laboratory standards.Culture being gold standard is unable to provide early results and necessarily 4-6 weeks are required for ultimate diagnosis.Newly administered GeneXpert MTB/RIF assay takes only two hours to provide the final results, distinguishes MTB and Mycobacteria other than tuberculosis and also provide rifampicin resistance simultaneously.*
Settings:*This descriptive study was carried out in Pakistan Medical Research Council TB Research Centre in collaboration with department of Chest Medicine King Edward Medical University/Mayo Hospital Lahorefrom December 2012 to March 2014.*
Objectives: *This study was undertaken to assess the value of GeneXpert in diagnosis of Mycobacterium tuberculosis complex in comparison with ZiehlNeelsen smear microscopy and to observe the additional diagnostic value of the technique.*
Design: *This is a descriptive study based on retrospective data.*
Results:*A total of 403 patients were included in present study of which 48.1% females and 51.9% males with mean age of 35.3±15.9. ZiehlNeelsen smear positivity for acid fast bacilli was 67.5%, GeneXpert positivity for TB remained 77.4% and culture positivity remained 85.1% for TB on culture. Sensitivity, specificity, negative predictive value and accuracy of GeneXpert were 90.1%, 98.3%, 62.6%, 91.3% are significantly higher as compared to smear which were 77.7%, 91.4%, 40.8% and 79.7% respectively. Positive predictive values were 99.7%, 98.2% for two techniques and there was no significant difference.*
Conclusion: *Diagnostic value of GeneXpert is significantly high as compared to ZiehlNeelsen smear microscopy and a useful tool in early diagnosis of tuberculosis.*
Key Words: *Prevalence of TB, Acid Fast Bacilli, Microscopy, LJ Medium, Rapid diagnosis*

I. Introduction

Tuberculosis (TB) is a transmissible bacteriological ailment triggered by *Mycobacterium tuberculosis* (MTB) and is still one of the biggest challenges for developing countries. TB spreads by inhaling minute droplets produced by coughing or sneezingfrom infected person(s).Even though TB is a curabledisease itstill manages to kill around 5000 people every day according to World Health Organization (WHO) [1]. This disease affects the most vulnerable community like poorest and malnourished individuals. Global incidence rate of TB is still rising 1% every year due to rapid increase of disease in Africa[1].Pakistan ranks 5thamongst the 22 high TBburdencountries. Prevalence of TB has been estimated to 350/100000 and mortality 33/100000 in Pakistan[2] while lesser mortality rate of 22/100000 is found to be in India[3].

Mycobacterium tuberculosis can be stained by using a simple histological stainingmethodcalled ZiehlNeelsen (ZN). The Acid fast bacilli in the smear (Mycobacterium tuberculosis) stains up bright red with background as blue.Making thisa popular method of choice for prompt diagnosis of MTBin the most of developing countries including Pakistan[4]. Main reasons forusing theZN smear microscopy is its low cost, high specificity, does not require sophisticated equipment or high laboratory standards. Provision resultscan be achieved usuallywithin two hours; however test is less sensitive and normallyrequires bacillihigh as 5000-10,000 per ml of specimen for the smear to become positive, however this test can't be used to distinguishbetween MTB and *Mycobacteria*only for tuberculosis[5]. Culture being gold standard is unable to provide early results and necessarily 4-6 weeks are required forultimate diagnosis. Relatively faster radiometric

(BACTEC) and non-radiometric methods like fluorescent labeled mycobacterium growth indicator test (MGIT) methods are being used and provide results in 7-10 days[5]both these methods are time consuming to some extent.

Serological tests used for TB diagnosis also were not useful for clinicians[6].Utilization of polymerase chain reaction (PCR) test for MTB diagnosis has been evaluated to appreciable extent and already initiated to influence clinical inspective microbiology[4].Efficacy of Lowenstein Jensen medium to detect MTB has been demonstrated as 10 viable bacilli per ml of specimen while PCR can detect as low as 10fg of bacilli which is equal to 2 bacilli per ml[4]. Newly administered GeneXpert MTB/RIF assay takes only two hours to provide the final results, distinguishes MTB and *Mycobacteria* other than tuberculosis and also provide rifampicin resistance simultaneously. WHO has strongly recommended this test for diagnosis of TB in various settings[7].

Aim of this study was to assess the value of GeneXpert MTB Rif Assay in diagnosis of *Mycobacterium tuberculosis* complex in comparison with ZiehlNeelsen smear microscopy and to observe the additional diagnostic value of the technique at a tertiary care setting.

II. Material And Methods

This descriptive study was carried out in Pakistan Medical Research Council TB Research Centre, in collaboration with department of Chest Medicine King Edward Medical University/Mayo Hospital Lahorefrom December 2012 to March 2014. A total of 422 subjects suspected to have active TB were included in present study.Varieties of pulmonary and extra-pulmonary specimens were collected and single specimen from each individual was included in present study however due to various kinds of errors on GeneXpert 17 (4.0%) specimens, and because of culture contamination 2 (0.5%) specimens did not give results; therefore these 19 specimens were not included in the final analysis.Patients were asked to submit 4-8 ml sputum specimen at PMRC TB Laboratory. Specimens were divided into two parts; half of the specimen was used for ZN smear and culture on Lowenstein Jensen (LJ) medium and another half was used for GeneXpert MTB Rif assay.GeneXpert was inducted in our system in November 2012; therefore retrospective data was analyzed from the period since its initiation at PMRC TB Research Center KEMU Lahore.

Direct and concentrated smears were prepared from each specimen using Petroff's method for decontamination and concentration[4]. A minimum of a100samples using oil immersion technique (oil fields)were observed before reporting a smear asnegative.Smearswhich wereconsidered as positive if the slide contained at least 3 acid fast bacilli (AFB) among 100 oil fields of microscope. Results were reported using the criteria of WHO/International Union of Tuberculosis and Lung Diseases (IUTALD)[4]. If no AFB was observed in 100 fieldsthan this was reported as negative, actual count was reported if 1-9 AFB seen per 100 high power fields, if 10-100 AFB were observe in 100 high power fields reported as 1^+, 1-10 AFB per high power field in at least 50 fields reported as $2^+$and more than 10 AFB per high power field in at least 20 fields reported as 3^+.Culture was considered positive if it contained only one colony. Actual colony count was reported if less than 50 colonies were present on LJ medium slant, if 50-100 colonies 1^+, 100-200 colonies 2^+ and more than 200 colonies per slant was reported as 3^+. Culture on LJ medium was considered as gold standard in present study.

Known positive and negative slides(Control Slides)were included with each run of ZN staining. An experienced microbiologist rechecked the smears for internal quality. ZN smears were also sent to the National TB Control Program for external quality assurance. LJ media were tested by inoculation of known American Type Culture control strain of H37RV. Random slants of LJ media inoculated with sterile distilled water were also incubated from each batch as negative controls.

Lysing reagent provided with GeneXpert MTB Rif Assay kit was mixed with specimen to be processed in a sterile conical tube.After vortex specimen is allowed to stand for 15 min and 2 ml of the mixture is added to the cartridge provided by manufacturer. This cartridge is provided with all the reagents required for nucleic acid amplification techniques described in the protocol[8].

III. Results

A total of 403 patients were included in this study, 194 (48.1%) of which were females and 209 (51.9%) were males.Male to female ratio of 1:1.08.Mean age of the subjects was found to be 35.3±15.9. Most of the subjects (40.9%) were in 21-35 years of age. Distribution of age, gender and region of study subjects are shown in table I.

Various kinds of pulmonary and extra-pulmonary specimens were included in this study. Highest proportion was covered by pulmonary specimens (394) consists of 387 (96.0%) sputum and 7 (1.7%) bronchial wash. Smear positivity was found to be 67.5% (273/403).GeneXpert positivity for MTB remained 77.4% (313/403) while 91 (22.6%) were negative on GeneXpert and 58 (14.4%) were negative for MTB on culture. Nature of thespecimen, ZN smear, GeneXpert and culture results distribution are shown in table II.

Sensitivity, specificity , negative predictive value (NPV) and accuracy of GeneXpert was found significantly higher as compare to ZN smear (non-overlapping confidence interval), while the positive predictive values (PPV) were same for both procedures as shown in table III.

Comparison of ZN smear and GeneXpert results for detection of MTB are shown in table IV.Among 130 ZN smear negative subjects 52 (40.0%) were found to be positive for MTB by GeneXpert.Out of 13 ZN smear positive, GeneXpert negative cases 9 (69.2%) consist of MTB while 4 (30.8%) consist of non-tubercle mycobacteria on the basis of para-nitro-benzoic acid test. On inquiring these 4 cases with clinician it was found that they do not have any clinical significance.

IV. Discussion

Early diagnosis of TB is necessary todisrupt the disease transmission chain. Although ZN smear positive patients are considered highly infectious and being focused by most of clinicians, smear negative patients are also reported to responsible for approximately 17% of transmission and its impact on public health could not be neglected[9].

Smear positivity in present study was found to be 67.5% and GeneXpert positivity for MTB remained 77.4% respectively,are not in agreement with a study recently published,which showed lower smear positivity of 53% by ZN stain and higher MTB positivity of 82% by GeneXpert[10].A wide range of 0-75% for ZN smear positivityhas been reported in earlier studies[11] however smear positivity washigh in present study may be due to inclusion of high suspicious cases. Previous studies in comparison with present settings have shown much lesser positivity[4,12].

Higher sensitivity (90.1%) and specificity (98.3%) of GeneXpertfor MTB detection has been reported as compared to ZN smear which was77.7% and 91.4% respectively in present study. A bit lower sensitivity of 67.6% for ZN smear has been reported in previous study[4]. Sensitivity and specificity of GeneXpert for detection of MTB was 98.0% and 98.3% respectively presented by manufacturer[8] is high as compared to the present study.A lower sensitivity of this technique has also been reported by another study under similar settings[10].Smear negative TB is more difficult to treat due to delay in reaching definite diagnosis, in such cases new diagnostic approaches could be fruitful in early diagnosis and prompt treatment, hence preventing the patients becoming infectious for others,as is shown in present study where 40.0% ZN smear negative subjects were found to be positive for MTB by GeneXpert. A study from South Africa also endorsed that ZN stained microscopy of sputum smears combined with detection of MTB by GeneXpert is most cost effective and accurate strategy for diagnosis of smear negative TB[13].Accuracy of GeneXpert for MTB detection (91.3%) was greater than ZN smear (79.7%) microscopy in present study is promising with former statement. Another advantage of this test is to preclude the drug susceptibility testing as it also provides the rapid rifampicin susceptibility along with MTB detection.

Culture using the LJ medium is yet method of choice for diagnosis of MTB and considered as gold standard in developing countries and its importance could not be neglected. Culture positivity of 85.1% in present study also proves the accuracy of culture but takes 4-8 weeks to produce results. This high prevalence is due to inclusion of highly suspicious and clinically significant cases in present settings. Rapid culture techniques like BACTEC and MGIT although reduced the detection time to 7-10 days hence equipment and running expenses are much high, more skilled personals, continuous monitoring for several days and further confirmation of positive cultures by making ZN smears are required[14]. In contrast although detection by GeneXeprt is bit expensive does not apply the conditions above, only one person can monitor fully automated system and provides result in two hours[8].

Mean age of subjects in present study was 35.3±15.9 years and female to male ratio of 1:1.08 are although different but comparable with another studythat showed mean age of 30.1years with female to male ratio 1:1.65[15]. This shows that the most of the TB patients (62.3%) lie in the most productive age 21-50 years in present study. Prevention from this noxious disease is obligatory to increase the detection rate by using individual or combined techniques which in turn can prevent greater economic loss.

In conclusion GeneXpert has shown significantly higher levels of sensitivity and specificity for diagnosis of MTB as compared to ZN smear microscopy and proved itself as an effective tool in initiation of early treatment.

References

[1]. Tuberculosis – the global burden. Available from the website: http://www.who.int/tb/publications/tb_global_facts_sep05_en.pdf
[2]. National Tb Control Program. Disease burden in Pakistan. Available from the website:http://125.209.94.75:7778/misdots/apex_040200.dad.misdots
[3]. TB statistics in "High Burden" countries 2012. Available from the website: http://www.tbfacts.org/tb-statistics.html
[4]. Munir M K, Anwer N, Iqbal R, Shabbir I, Nosheen S. Diagnosis of tuberculosis:Molecular versus conventional method. Pak J Med Res. 2011; 50(2): 50-4.
[5]. Yam WC. Recent advances in rapid laboratory diagnosis of tuberculosis. MWD Bull 2006; 11: 6-7.
[6]. Munir M K,Shabbir I, Iqbal R, Khan S U, Syed Z A. Diagnosis of extra-pulmonary tuberculosis: Conventional versus newer methods. Pak J Chest Med. 2009; 15(3): 11-14.
[7]. World Health Organization. Tuberculosis diagnostics Xpert MTB/RIF Test. Fact Sheet. May 2012. Available from the website: http://www.who.int/tb/features_archive/factsheet_xpert_may2011update.pdf

[8]. Xpert MTB/RIF. Two-hour detection of MTB and resistance to rifampicin. AvailablefromURL:http://tbevidence.org/documents/rescentre/sop/XpertMTB_Broch_R9_EU.pdf
[9]. Mostaza J L, Garcia N, Fernandez S, Bahamonde A, Fuentes M I, Palomo M J. Analysis and predictor of delay in suspicion and treatment among hospitalized patients with pulmonary tuberculosis. An Med Interna. 2007; 24(10): 478-83.
[10]. BuchelliRmirez H L, Gracia-Clemente M M, Alvarez-Alvarez C, Palacio-Gutierrez J J, Pando-Sandoval A,Gagatek S, Arias-Guillen M, Quenzada-Loaiza C A, Casan-Clara. Impact of the Xpert MTB/RIF molecular test on the late diagnosis of pulmonary tuberculosis.Int J Tuberc Lung Dis. 2014;18(4): 435-37.
[11]. Kamboj S S, Goel M M, Tandon P I. Correlation study of histopathology and bacteriology in patients of tubercular lymphadenitis. Indian J Chest Dis Allied Sci. 1994; 36: 187-91.
[12]. Rehman S, Iqbal R, Munir M K, Salam A A, Saeed S. Incremental yield of submitting three sputum specimens for the diagnosis of pulmonary tuberculosis. Pak J Med Res. 2013; 52(2): 35-8.
[13]. Theron G, Pooran A, Peter J. Do adjunct tuberculosis tests, when combined with Xpert MTB/RIF, improve accuracy and the cost of diagnosis in a resource poor settings? EurRespir J. 2012; 40:161-68.
[14]. Siddiqui S H, Rusch-Gerdes S. Mycobacterium growth indicator tube (MGIT) culture and drug susceptibility demonstration projects. Available from URL: http://www.finddiagnostics.org/export/sites/default/resource-centre/find_documentation/pdfs/mgit_manual_nov_2007.pdf
[15]. Munir M K, Anwer N, Iqbal R, Nosheen S, Rehman S, Salam A A. Comparision of Genotype MTBDR*plus* testing kit with conventional method for drug susceptibility testing of isoniazid and rifampicin in tuberculosis patients. Pak J Med Res. 2014; 53 (2): 25-30.

Table I: Gender, Age and Region wise Distribution of Patients

Characteristics		**N = 403**	**%**
Gender	F	194	48.1
	M	209	51.9
Age 35.1±15.9 (15 – 90)	<= 20	81	20.1
	21 - 35	165	40.9
	36 - 50	84	20.8
	51 - 65	60	14.9
	66+	13	3.2
Region/Division	Lahore	327	81.1
	Gujranwala	54	13.4
	Bahawalpur	6	1.5
	Faisalabad	7	1.7
	Multan	6	1.5
	KPK	3	0.7

Table II: Results of Specimens on ZN smear, GeneXpert and Culture.

Characteristics		N = 403	%
Specimen	Sputum	387	96.0
	Br W	7	1.7
	Pus	4	1.0
	CSF	1	0.2
	Fluid	1	0.2
	Lymph Node	1	0.2
	Pleural Fluid	1	0.2
	EP	1	0.2
ZN Smear	Scanty	15	3.7
	1+	125	31.0
	2+	66	16.4
	3+	67	16.6
	N	130	32.3
GeneXpert	VL	20	5.0
	L	93	23.1
	M	140	34.7
	H	59	14.6
	N	91	22.6
Culture	P	345	85.6
	N	58	14.4

VL= Very Low, L= Low, M= Medium, H= High, P=Positive, N=Negative

Table III: Sensitivity and Specificity Comparison of ZN smear and GeneXpert. (N=403)

Factor	ZN Smear		GeneXpert	
	Value	95% CI	Value	95% CI
Sensitivity	77.7	73.6 - 81.8	90.1	87.2 - 93.0
Specificity	91.4	88.7 - 94.1	98.3	97.0 - 99.6
PPV	98.2	96.9 - 99.5	99.7	99.2 - 100.0
NPV	40.8	36.0 - 45.6	62.6	57.9 - 67.3
Accuracy	79.7	75.8 - 83.6	91.3	88.5 - 94.1

Table IV: Distribution and findings on ZN smear and GeneXpert

ZN Smear Result	GeneXpert					Total
	VL	L	M	H	N	
Scanty	1	7	6	0	1	**15**
1^+	4	46	60	7	8	**125**
2^+	0	6	39	17	4	**66**
3^+	1	3	28	35	0	**67**
Negative	14	31	7	0	78	**130**
Total	**20**	**93**	**140**	**59**	**92**	**403**