Short Communication

Validity of Concentrated Sputum Microscopy versus Rapid Gene Xpert MTB/RIF in Case Detection of Presumptive Pulmonary Tuberculosis

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Abstract

Background: The diagnosis of pulmonary tuberculosis in Iraq started with direct sputum microscopy. The case detection rate is still below target level. Advance WHO-approved rapid diagnostic tests (Gen Xpert MTB/RIF) is now available for diagnosis. **Objectives:** To verify the validity and predictive value of direct and concentrated sputum microscopy in relation to Gen Xpert test for newly presumptive patients. **Materials and Methods:** A cross sectional study of 1175 patients attended the Respiratory and Chest Consultation Clinic in Najaf governorate, middle of Iraq. All patients suspected of having pulmonary TB submitted at least two early morning sputum specimens to detect acid fast Mycobacterium bacilli. One sputum sample from each patient was sent for Gen Xpert testing for both positive and negative result of smear microscopy. Direct and concentrated sputum smear with Ziehl Neelsen staining were prepared on the same specimen. The data was analyzed to estimate the accuracy rate, sensitivity and specificity, in addition to predictive value for positive and negative test. **Results:** From 1175 patients, only 396 completed their sputum submission for both sputum microscopy and molecular Gen Xpert per time. The sensitivity of direct smear microscopy versus concentrated sputum was 65% and 69.1% respectively with no significant difference. The specificity of direct smear result was 80.1% versus 76.1% for concentrated sputum smear. **Conclusion:** Sensitivity of both direct and concentrated sputum microscopy showed insignificant low detection rate. The direct smear test was found lightly more specific than concentrated smear.

Keyword: Pulmonary tuberculosis, Sputum Microscopy, Gen Xpert MTB/RIF.

Introduction

The Nation Tuberculosis Control Program in Iraq (NTP) was established in1989. It was supported by WHO to start Directly Observed Therapy strategy (DOTS) in 1998 which was implemented in the year 2000 all over the country. Tuberculosis is still a killer of targeted age group from younger population. The program included the services of case detection and treatment at the level of primary health care districts and their related PHC centers. [1] The case detection depends on the accuracy of several diagnostic feasible, cost-effective methods on presumptive patients presented with symptoms of unexplained productive cough for three weeks or more referred to the respiratory and chest diseases consultation clinic found in each governorate. [2] The level of certainty of the diagnosis depends on bacteriological confirmation and clinical diagnosis of suspected cases. The clinically diagnosed case is one which is not fulfill the requirements of bacteriological confirmation depending on clinical findings besides X ray abnormalities of histological and extra pulmonary case management without bacteriological confirmation. [3,4] Bacteriologic-al confirmation refers to identifycation of *Mycobacterium tuberculosis* by sputum microsc-

opy, culture or other new advanced molecular methods. In the national program, all patients presumptive to have pulmonary tuberculosis (PTB) should give at least two sputum specimens for microscopically examination in a qualified laboratory. The submitted sputum should include at least one early morning specimen. [1,5] The program recommends that all persons with radiological chest abnormalities should submit his sputum for smear microscopically examination. The sputum positive case of PTB refers to presence of at least one acid fast bacillus (AFB) in at least one specimen of sputum in a quality -assured laboratory, or culture positive or WHO recommended rapid diagnostic tests like GenXprt MTB/RIF. Sputum culture is still difficult to perform this locality. [1,6] GeneXpert MTB/RIF was approved by WHO in 2010 for detecting both M. *tuberculosis* detect and rifampicin resistance status in one setting. It is a molecular fully automated and cartridge-based real-time PCR. [1] The test plays an important role in improving early management of TB cases and strengthen the control measures. [7,8] It was hypothesized that concentrated sputum sear microscopy might increase their validity in detection of AFB suspect cases. This study aims to verify the sensitivity and specificity of the direct and concentrated sputum in comparison with recently applied GenXper molecular technique in Iraq. [3] This study aims to verify the validity and predictive value of direct and concentrated sputum microscopy in relation to Gen Xpert test for newly presumptive patients.

Materials and Methods

A cross-sectional diagnostic study conducted to verify the validity of direct and concentrated sputum microscopy in comparison with golden highly sensitive and specific Gen Xpert in detecting TB cases among persons referred with unexplained cough to consultation clinic of Respiratory and Chest diseases in Najaf governorate, Jan 2020 through May 2024. From 1175 referred patients, 470 suspected to have pulmonary tuberculosis (PTB). Only 396 submitted three samples of sputum and missing of 74 patients including 24 children below 15 years of age. The sample consisted of all presumptive PTB patients 15 years of age and more. All patients suspected of having pulmonary TB submitted at least two early morning sputum specimens to detect acid fast Mycobacterium bacilli by direct smear and concentrated specimen method to increase density of the bacilli. One sputum sample from each patient was sent for Gen Xpert testing to confirm the true positive and true negative patients. The acid-fast bacilli examination done after Ziehl Neelsen staining. The sensitivity, specificity, and predictive value for positive and negative result of AFB microscopy were calculated in comparison with GeneXpert MTB/RIF results as a golden diagnostic test. The sensitivity indicated the ability of sputum microscopy to give correct diagnosis. which means the percentage of patients with positive test. While specificity is the ability of the test to give correct diagnosis of negative individuals as healthy, or the proportion of healthy people whose test results are truly negative.

Statistical analysis

Data analysis was applied by SPSS version 27. Descriptive statistics were estimated for some variables including, age group, sex, TB symptoms and X-ray finding in addition to calculation of sensitivity, specificity, and predictive values of sputum microscopy compared with results of GenXpert MTB/RIF test.

Ethical Approval

The College of Medicine at the University of Al-Kafeel ethical committee approved this study's

ethical approval, obtaining verbal consent from each patient and control. A local ethics committee reviewed and approved the subject information and consent form.

Results

The totally referred patients suspected to have tuberculosis (TB) were 1175, from whom 470 (60%) patients categorized as presumptive pulmonary TB (Figures 1). The study sample consisted of 260 (55.3%) males and 210 (44.7%) females. The higher proportion of suspected pulmonary TB were found in age groups (15-34 years). Most of the patients were referred from private clinics ,436 (92.8%) presented with symptoms and 416 (88.5%) had x-ray TB findings including two HIV positive individuals (Table 1). Both direct and concentrated sputum smear microscopy revealed low sensitivity (65% versus 69.1%), and good specificity (80.1% versus 76.1%) respectively compared with the results of GenXpert test. The predictive value of direct smear microscopy was 80.3% for positive test and 64.7% for negative one (Table 2). While predictive value of concentrated sputum microscopy was 78.4% for positive test, and 66.3% for negative one (Table 3). The paired results of positive and negative sputum smear microscopy and Gen Xpert tests showed statistically significant difference (P=0.00001).

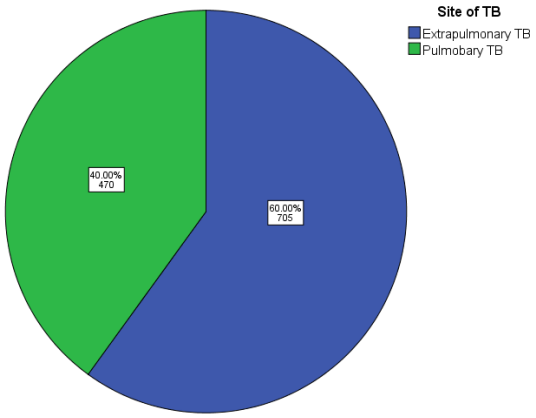

Figure 1: Distribution of referred patients by site of TB

Table 1: Personal Characteristics of the referred presumptive pulmonary TB patients

Patient characteristics		Frequency (N=470)	Percent
Age group (years)	0-4	2	0.4
	5-14	22	4.6
	15-24	91	19.4
	25-34	96	20.4
	35-44	60	12.8
	45-54	70	14.9
	54-64	54	11.5
	65+	75	16.0
Sex	Female	210	44.7
	Male	260	55.3
Referral source	Private	254	54.0
	Public	137	29.2
	Self	79	16.8
Symptoms	No	34	7.2
	Yes	436	92.8
Type of patient	D(S-)(ND)	2	0.4
	F(S+)	2	0.4
	N(EP)	6	1.3
	N(S-)(ND)	98	20.9
	N(S+)	323	68.7
	R(S-)(ND)	12	2.6
	R(S+)	27	5.7
HIV test	Negative	468	99.6
	Positive	2	0.4
X ray Finding	No TB sign	54	11.5
	With TB sign	416	88.5

Table 2: Results of direct sputum smear microscopy versus GenXpert examination

Sputum examination (N=396)			Gen Xpert		Total	McNamar P value
			Positive	Negative		
Direct Sputum microscopy	+ ve	Count	143	35	178	0.00001
		%	65.0%	19.9%	44.9%	
	- ve	Count	77	141	218	
		%	35.0%	80.1%	55.1%	
Total		Count	220	176	396	
		%	100.0%	100.0%	100.0%	

Sensitivity of direct sputum microscopy v GenXpert 143/220x100 =65%
Specificity of direct sputum microscopy v GenXpert 141/176 x100 =80.1%
Positive predictive value of direct sputum microscopy v GenXpert 143/178 x100 =80.3 %
Negative predictive value of direct sputum microscopy v GenXpert 141/218 x100 =64.7 %

Table 3: Results of concentrated sputum smear microscopy versus GenXpert examination

Sputum examination (N=396)			Gen Xpert		Total	McNamar P value
			Positive	Negative		
Direct Sputum microscopy	+ ve	Count	152	42	194	0.00001
		%	69.1%	23.9%	49.0%	
	- ve	Count	68	134	202	
		%	30.9%	76.1%	51.0%	
Total		Count	220	176	396	
		%	100.0%	100.0%	100.0%	

Sensitivity of concentrated sputum microscopy v GenXpert 152/220x100 =69%
Specificity of direct sputum microscopy v GenXpert 134/176 x100 =76.1%
Positive predictive value of direct sputum microscopy v GenXpert 152/194 x100 =78.3 %
Negative predictive value of direct sputum microscopy v GenXpert 134/202 x100 =66.3 %

Discussion

The Iraqi TB control program still recommend sputum microscopy as initial tool to detect the acid-fast bacilli in suspected patients with tuberculosis in a qualified reference laboratory. The current study aimed to clarify the validity of the contracted specimen of sputum to increase the density of bacteria through well prepared smear. [3] The smear findings were compared to the recently applies molecular rapid technique recommend by WHO. GenXpert test is highly specific and sensitive methods for bacteriological purpose and identifying rifampicin resistance status. [1] The sputum microscopy was confirmed to be low sensitive in detection of the AFB positive smears in several studies. The sensitivity of sputum microscopy was generally good but the current study reveals a low sensitivity and specificity rate in both direct and concentrated (65% and 69.15 respectively) which necessitate depending initially on the rapid GentXpert test.

The rapid Gen Xpert diagnostic test is accurate and more valid as diagnostic test of both pulmonary and extrapulmonary TB. But it is now not widely applicable and remains a challenge in this locality where the available resources are limited which requires more specific laboratories and expertise personnel. The applying of rapid molecular Gen Xpert technique[3,9,10]. It is recommended to start with sputum microscopy to screen all suspect TB cases.

While concerning the feasibility of sputum microscopy in current situation, it is important to consider the demographic factors like age and sex, and HIV infection which increase the patient's risk's exposure to TB bacilli[12]. This study estimated high proportion of TB infection among young and active strata of population with approximate male and female risk of infection. This verifies the continuous increasing prevalence of the disease after underestimation during COVID-19 crises[12-14]. Taking at least one early morning sputum with more quality assured laboratory will increase the validity of sputum microscopy. High proportion of referred patients had symptoms (92.6%) which would consist the results of low sensitive sputum microscopy.

Diagnostic research suggested that the sensitivity of AFB smear microscopy varies between 20% to 80% [13]. Steingart KR *et.al* in addition to Churchyard G J *et.al* consisted that the sensitivity of smear microscopy was only 46% [15,16]. The current study, we could only diagnose approximately 65% to 69% % of suspected TB cases by sputum smear microscopy compared to GenXpert results. [16-17] The sensitivity of smear microscopy showed statistically difference data due to difference in the quality of sample, preparing processing and experience and training of the laboratory staff on sputum microscopy. [17,18] The septicity and positive predictive value of sputum microscopy were relatively high in comparison with the rapid molecular test which indicates its role in detection of TB cases in spite of its low sensitivity because of its feasibility, cost-effectiveness and the resources made available.

The sensitivity of concentrated sputum showed insignificant difference from direct sputum microscopy which makes the direct smear technique more practical within the current situation. [15,19]

The Gen Xpert assay was applied as a golden test because of its availability and high validity similar to validity of sputum culture (99%-100% sensitivity) in addition to difficulty in culture application. This study verified a good specificity for direct sputum microcopy, that mean good ability to identify sputum negative pulmonary TB better than concentrated specimens. [18,20]

Some evidence based results regarding staining sputum method, sensitivity and specificity in Ziehl–Neelsen staining methods method were 76.74% (95% CI 68.42% -85.06%) and 93.42% (95% CI: 90.29%- 96.54%) respectively. Staining method was still applicable, consisted by measuring sensitivity and specificity in some published studies that used Ziehl–Neelsen were found 76.74% (95% CI 68.42% -85.06%) and 93.42% (95% CI: 90.29%- 96.54%) respectively. This study measured good predictive value in direct and concentrated methods (80%.3 in both) [16]. The importance of sputum smear microscopy consisted in pooled sensitivity of sputum smear microscopy of 75.12% with 25% under-diagnosis error. Sputum microscopy was used for follow up of teared patients not the Gen Xpert assay. [20]

Conclusion

Sensitivity of both direct and concentrated sputum microscopy showed insignificant low detection rate. The direct smear test was found lightly more specific than concentrated. Direct sputum smear is more applicable and feasible in a quality assurance laboratory because of difficulty and short resources of Gen Xpert test and culture facilities.

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