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Comparison of GeneXpert against Light-Emitting Diode Fluorescent Microscopy for the Diagnosis of Pulmonary Tuberculosis in Addis Ababa, Ethiopia

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Abstract

Background: Tuberculosis remains a global health problem despite availability of effective tools. Globally, there were 40% of the 10+ million patients with TB did not get diagnosed or notified. The study was aimed to compare the diagnostic performances of iLED-FM and GeneXpert test for the diagnosis of PTB in Addis Ababa, Ethiopia.

Methods: Facility-based cross-sectional study was conducted on a total of 286 sputum samples collected from health centers and hospitals clients with presumptive TB from December 2016 to March 2017. Kappa value, Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of iLED-FM and GeneXpert were calculated against the gold standard.

Results: The sensitivity, specificity, PPV and NPV of iLED-FM was 80.15%, 95.48%, 93.75% and 85.06%, for GeneXpert 88.55%, 92.90%, 91.34% and 90.57%, respectively. Kappa value of iLED-FM was 0.765 and 0.817 for GeneXpert. Out of 55 HIV positive presumptive TB patients enrolled in the study, 19 (34.55%) were sputum smear positive by iLED-FM. However, 24 (43.64%) TB cases were detected by GeneXpert.

Conclusion: The sensitivity of Xpert MTB/RIF assay was better than iLED-FM in the diagnosis of PTB. It should be implemented as primary diagnostic test in areas where overlapping synergy of TB and HIV/AIDS is high.

Keywords: Addis Ababa; Ethiopia; GeneXpert; Light-emitting diode; Microscopy; Tuberculosis

Abbreviations: AIDS: Acquired Immuno Deficiency Virus; CI: Confidence Interval; HIV: Human Immuno-Virus; LED-FM: Light Emitting Diode Fluorescent Microscopy; L-J: Lowenstein-Jensen Media; MTB: *Mycobacterium tuberculosis*; MGIT: Mycobacterial Growth Indicator Tube; MDR-TB: Multi-Drug Resistance Tuberculosis; PPV: Positive Predictive Value; NPV: Negative Predictive Value; SOPs: Standard Operating Procedures; SPSS: Statistical Package for Social Science; TB: Tuberculosis; WHO: World Health Organization

Introduction

Despite availability of effective tools and the main advances in TB diagnostic technologies over the world, ZN-stained direct sputum smear microscopy remains the most widely used diagnostic method available in most health-care laboratories of developing countries [1]. However, it has low sensitivity compared to auramine-O stained sputum smear examined by LED-FM and Mycobacterium tuberculosis (MTB) culture [1,2]. It is a responsibility for diagnosing of 40% of the 10+ million patients with TB did not get diagnosed or notified in globally [3,4].

Conventional culture has been estimated to detect 10 to 100 viable MTB per milliliter of sputum [3]. But this method requires infrastructures, costly, technical demanding, time taking for result delivery and not suitable for patient management [1,2].

Recently, molecular diagnostics are increasingly being promoted as TB diagnostic tools due to rapid turnaround time, high sensitivity and specificity of the techniques [2,5]. Consequently, WHO recommend GeneXpert to be used as an initial diagnostic test in individuals suspected of Multi-Drug resistance tuberculosis (MDR-TB), extra PTB, HIV-associated TB and children suspected of TB. Children and HIV/AIDS patients are immune compromised and unable to spout out productive sputum. Hence, misdiagnosis is common by ZN-techniques in these patient groups [6].

In Ethiopia, most health facilities have limited capacities to diagnose TB. For instance, in 59% health facilities in Ethiopia, the only available diagnostic test was sputum smear microscopy, 6% have chest x-ray and 2% have GeneXpert. According to center for strategic and international studies, nearly 48% of all active TB cases would have been missed while screened by sign and symptoms [7]. Hence, the need to expand and utilize different diagnostic modalities in different context of Ethiopia plays great role in increasing case detection and control of TB.

In study setting, there is a direction to utilize GeneXpert for all TB presumptive patients' diagnosis. However, in Ethiopia, only 2% health facilities have GeneXpert machine to diagnose TB [6]. As a result, access limits its utilization for all TB presumptive patients. Hence, there is greater need of other alternative diagnostic methods particularly in study area and other similar settings.

Even though WHO recommended LED-FM be used as an alternative to ZN-stained sputum smear microscopy for TB case detection, the

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diagnostic performance of LED-FM was not evaluated in study setting [1]. Therefore, this study was aimed to compare the performance of auramine O-stained direct sputum smear examined by LED-FM against GeneXpert test for the diagnosis of PTB in Addis Ababa, Ethiopia.

Materials and Methods

Study design and setting

A facility based cross-sectional study design was conducted from December 2016 to March 2017 to compare the diagnostic performances of iLED-FM and GeneXpert test for the diagnosis of PTB in Addis Ababa, Ethiopia. Based on 2007 census conducted by Ethiopian national statistics authorities, Addis Ababa has 10 sub city and 116 Woreda (the lowest District of City Administration) with an estimated total population of 3,384,569 and annual growth rate of 3.8% [8]. In the city, there are more 896 public and private health facilities, of which 101 public and 52 privates are being delivering TB diagnostic services [9]. Sputum specimens are referred though postal system to regional laboratory or lead hospitals where Xpert MTB/RIF assay machines are available and result sent back to referred health facilities through postal system of the existing national referral linkage map of testing network.

Sample size determination and sampling techniques

The sample size was determined using single population proportion formula considering the crude prevalence of TB in Ethiopia as 19% for convenience [10]. Accordingly, a total of 286 sputum samples were collected from TB presumptive patients from private and public health facilities of Addis Ababa during the study period were included.

Laboratory testing procedures and sputum specimen management were performed according to stated Standard Operating Procedures (SOPs) of LED-FM sputum smear microscopy, GeneXpert and L-J sputum culture [3,6]. Seven to ten milliliters of sputum samples (2-4 ml for GeneXpert and the remaining for culture) were collected in two separate falcon tubes. Strict procedures were followed during reagents preparation, sputum smears preparation and staining. The qualities of the prepared reagents were checked by staining with known positive and negative quality control sputum smear slides. After quality checks of the reagents, smears prepared from direct sputum were stained by auramine-O, air dried and examined by LED-FM [11-13].

Before inoculation on L-J culture media, sputum samples were decontaminated by N-Acetlyl-L-Cysteine-Sodium Hydroxide (NALC-NaOH) [3]. The sterility of the prepared culture media were checked by incubating in an incubator for 48 h at 37°C to see if there is no over growth of other contaminants [6]. After quality check of the prepared culture media, 0.1ml of decontaminated and distilled water re suspended sputum sample was inoculated on (L-J) media [3].The other non-decontaminated sputum samples in the second falcon tubes were analyzed by GeneXpert test [6].

Sputum processing for smear preparations and liquefaction for GeneXpert test were performed in bio-safety level-2 laboratories dedicated for MTB work with an appropriate ventilation system. Decontamination and inoculation on L-J culture media were performed in bio-safety level-3 and accesses to the room were restricted when the work was in progress. Appropriate protective equipment including gowns, gloves and respirator masks (N-95) were used while handling specimens [3]. Before removing from laboratory, all tubes with the culture media were sterilized using an autoclave at 121°C for 15 min to the acceptable level of sterilization [3].

Data management and statistical analysis

The results of each test were entered to the excel sheet, checked for completeness, consistency and transferred to SPSS version 20 (SPSS Inc. Chicago, USA) software for analyses. The sensitivity, specificity, PPV and NPV of LED-FM and GeneXpert were calculated against the gold standard.

Results

Socio-demographic characterstics of study participants

From the total of 286 PTB presumptive patients enrolled in the study, 117 (40.91%) were female. The average age of the study participants were 35.13 year with the minimum and maximum age of 18 and 80, respectively. Of 286 study participants, 172 (60.14%) were tested for HIV/AIDS of whom male accounted 96 (55.81%). Sero-positivity rate among those tested for HIV/AIDS were 55 (32%); 26 (27.08%) among male and 29 (38.16%) among female as illustrated in Table 1, of 55 sero positives, 24 (43.6%) were positive for TB by GeneXpert test but 19 (34.5%) were positive by LED-FM as presented in Table 2.

On the other hand, treatment categories of patients screened for MTB were reviewed from the requested paper. Of total the PTB presumptive patients enrolled in the study, 148 (51.75%) were new cases, 73 (25.52%) relapse, 22 (7.70%) treatment failure, 1 (0.35%) defaulter and 41(14.34%) have no recorded previous treatment history.

Comparison of direct LED-FM and culture results

From a total of 286 sputum samples analyzed by culture and LED-FM, 105 (36.71%) were positive by both methods; 7 (2.45%) were smear positive by LED-FM but culture negative; 148 (51.20%) were negative by both methods and 26 (9.09%) were culture positive but smear negative by LED-FM (Table 3). The sensitivity, specificity, PPV and NPV of LED-FM was 80.15% (95% CI: 72.29%-88.61%), 95.48% (95% CI:

Age group in years	Male (%)	Female (%)	Total (%)
18-27	43 (25.44)	41 (35.04)	84 (29.37)
28-37	62 (36.69)	35 (29.91)	97 (33.92)
38-47	31 (18.34)	23 (19.66)	54 (18.88)
48-57	15 (8.88)	12 (10.26)	27 (9.44)
58-67	12 (7.10)	2(1.71)	14 (4.90)
≥ 68	6 (3.55)	4 (3.42)	10 (3.50)
Total	169 (59.09)	117 (40.91)	286 (100)
HIV Status			
Positive	26 (26.08)	29 (38.16)	55 (31.98)
Negative	70 (72.92)	47 (61.84)	117 (68.02)
Total	96 (100)	76 (100)	172 (100)

Table 1: Socio-demographic characterstics of study participants by age, sex and HIV-status, Addis Ababa, Ethiopia.

	HIV status		
LED-FM	**Positive**	**Negative**	**Total**
Smear positive	19	64	83
Smear negative	36	53	89
GeneXpert			
MTB detected	24	50	74
MTB not detected	31	67	98
MTB culture			
Positive	22	47	69
Negative	33	70	103

Table 2: HIV-results and TB results with diagnostic methods, Addis Ababa, Ethiopia.

Methods to be Evaluated		Mycobacterium Culture		
		Positive (%)	Negative (%)	Total (%)
iLED-FM	Positive (%)	105 (36.71)	7 (2.45)	112 (39.16)
	Negative (%)	26 (9.09)	148 (51.75)	174 (60.84)
	Total	131 (45.80)	155 (54.20)	286 (100)
Xpert MTB/RIF® assay	Positive	116 (40.56)	11 (3.85)	127 (44.41)
	Negative	15 (5.24)	144 (50.35)	159 (59.59)
	Total	131 (45.80)	155 (54.20)	286 (100)

Table 3: Comparison of LED-FM, GeneXpert and culture results, Addis Ababa, Ethiopia.

90.92%-98.17%), 93.75% (95% CI: 87.86% to 96.88%) and 85.06% (95% CI: 80.11% to 88.94%), respectively. The diagnostic yeild and accuracy of the test method was 36.71 and 39.16, respectively. The measure of agreement between the LED-FM and reference standard results were substantial with kappa coeffient (k=0.765) and p-value (<0.001).

Comparison of GeneXpert and culture results

From the total of 286 sputum samples analyzed by both GeneXpert and L-J culture, 116 (40.56%) were positive by both methods,11(3.85%) were positive by GeneXpert but culture negative, 144 (50.35%) were negative by both GeneXpert and MTB culture while 15 (5.24%) were negative by GeneXpert but culture positive (Table 3). The final results of both GeneXpert and culture were provided to the physician of the patients for the treatement decision. The sensitivity, specificty, PPV and NPV of GeneXpert was 88.55% (95% CI: 81.82% to 93.45%), 92.90% (95% CI: 87.66% to 96.40%), 91.34% (95% CI: 85.60% to 94.92%) and 90.57% (95% CI: 85.61% to 93.93%) respectively. The diagnostic yeild and accuracy of the test method was 40.56% and 44.41%, respectively. The measure of agreement between GeneXpert and culture results were perfect with kappa coeffient (k=0.817) and p-value (<0.001).

Comparison of LED-FM, GeneXpert and culture results

As presented in Table 3 from the total of 286 sputum samples examined by LED-FM, GeneXpert and culture, 105 were positive by LED-FM and culture. However, 116 cases were positive by GeneXpert and culture.

Discussion

In the present study, the diagnostic performances of auramine O-stained sputum smear examined by LED-FM and GeneXpert test were compared against MTB culture as a gold standard. The sensitivity, specificity, PPV value and NPV of GeneXpert was 88.55%, 92.90%, 91.34% 90.57%, respectively and LED-FM was 80.15%, 95.48%, 93.75%, 85.06%, respectively.

The sensitivity of GeneXpert in the present study was similar to meta-analyses reports of 22 studies pooled sensitivity 88% of WHO implementation manual 2014 [11]. In addition, it was in line with the sensitivity 90.1% reported from Pakistan [14]. However, it was lower compared to 97.14% reported from Egypt [15]. The difference might be due to from the sample size. Study in Egypt was conducted on 40 sputum samples but the present study, 286 sputum samples were analyzed.

The sensitivity of GeneXpert in current study was higher than the sensitivity 83.9% reported from Thailand [16]. The study carried out in Thailand was on smear negative PTB while the present study include both smear positive and negative sputum samples which might attributed to the difference. The sensitivity of the present study was higher than the study conducted in Kosovo, sensitivity 82.3% [17]. The difference might result from the sample size used in the study. Study conducted in Kosovo, 116 sputum samples were analyzed.

The specificity of GeneXpert in the present study was lower than the specificity 98.3% reported from Pakistan [14]. The difference might be due to sample size, study setting and sample type. In study of Pakistan, a total 403 samples were analyzed of which some are extra pulmonary specimens. The specificity of the current study was higher compared to specificity 86.4% of study conducted in Thailand [18]. The difference might be due to small sample size (109) and the gold standard used. The in study of Thailand, Mycobacterium Growth Indicator Tube (MGIT) was used as the gold standard, some of which grew non-tuberculosis Mycobacteria.

The specificity of GeneXpert in the current study is higher compared to 75% of study conducted in Egypt [19]. This might be attributed to study population and sample size. The present study includes all treatment categories and the sample size 286. But the study in Egypt includes retreatment cases and sample size of only fifty eight.

The sensitivity of LED-FM in the present study was higher than 73.2% reported from Kenya [19]. The study conducted in Kenya used larger sample size (1394) and the prevalence of TB in general population may vary. Our study result showed almost comparable sensitivity with WHO LED microscopy policy implementation 84% and 84.7% reported from Cape Town, South Africa [1,20,21]. Meta analyses conducted on 45 studies indicated the sensitivity LED-FM ranged from 52% to 97% which encompass the present study sensitivity [22].

In this study, the specificity of LED-FM 95.48% was comparable to study reported from Kenya specificity 96.7% [23]. The specificity of our study finding was relatively comparable with WHO LED microscopy policy implementation 98% and 98.9% of study reported from Cape Town, South Africa [1].

Conclusion and Recommendation

In this study, the sensitivity of GeneXpert is better than LED-FM for the diagnosis of PTB. It should be implemented as primary diagnostic test in areas where overlapping synergy of TB and HIV/AIDS is high. The implementation of LED-FM should be supplemented by GeneXpert in areas where the prevalence of HIV/AIDS is high. For sero-negative presumptive TB patients LED-FM should be implemented as diagnostic test of PTB in Addis Ababa, Ethiopia.

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