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Evaluation the role of conventional and Xpert MTB/RIF assays as point-of-care tests of *Mycobacterium tuberculosis* infections, especially during the COVID-19 pandemic in Menoufia, Egypt

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Abstract

Background

Tuberculosis (TB) is a destructive pulmonary disease, which was the most fatal infectious disease in the world for many years before the COVID-19 outbreak. During pandemic, COVID-19 was the main concern in every clinic and there were overlapping respiratory diseases resulting in delaying of the diagnosis and treatment of TB. Xpert MTB/RIF assay and Ziehl–Neelsen (ZN) stain are the most commonly used point-of-care test (POCT) assays for TB that were endorsed by WHO allowing a quick treatment turnaround time of a few minutes or hours, hence avoiding patient loss to follow-up. The aim of the study was to evaluate the role of Xpert MTB/RIF as a POCT for early, rapid, and accurate diagnosis of pulmonary and extrapulmonary TB during the COVID-19 pandemic and its role for exclusion of non-mycobacterial TB infections and to evaluate the proportion of patients with active pulmonary TB among COVID-19 patients and to study the difference of some inflammatory markers between patients with COVID-19, patients with pulmonary TB, and patients infected by both TB and COVID-19.

Patients and methods

This study was conducted from February 2018 to December 2021 (including the peak period of COVID-19 on 835 suspected TB patients (629 + 206 suspected COVID-19 patients six of them were proved pulmonary TB). Patients were from Shebin El-Kom Teaching Hospital and Chest hospital, Menoufiya). All 835 (pulmonary and extrapulmonary samples) patients were tested by gene Xpert MTB/RIF including 441 of them tested by ZN only. For detection of sensitivity, specificity positive predictive value (PPV), negative predictive value (NPV), and accuracy we selected 103 samples who were tested by the three methods (gene Xpert MTB/RIF, ZN staining, and culture on LJ media). For studying the difference of some inflammatory markers between patients with COVID-19, patients with pulmonary TB, and patients infected by both TB and COVID-19, 206 patients who were suspected of comorbid TB and COVID-19 during the pandemic were divided into three groups: group I positive for TB and COVID-19 ($N = 6$), group II positive COVID-19 only ($N = 100$), and group III were positive pulmonary TB only ($N = 50$) (NB: 50 patients were excluded due to incomplete data). Blood samples were taken for complete blood count, erythrocyte sedimentation rate, malondialdehyde, interleukin-6, C-reactive protein, D-dimer, ferritin, lactate dehydrogenase, calprotectin, and procalcitonin. Nasal swabs were needed for confirmation of COVID-19 by PCR.

Results

Compared with culture as a gold standard, sensitivity, specificity, PPV, and NPV for ZN smear were 77.1, 100, 100, and 53.8%, respectively. As regards the results of XPERT MTB/RIF assay, from the 103 samples examined, 89 (86%) were positive and 14 (14%) were negative. Eight false-positive results were recorded, compared with culture. The sensitivity was 98.8%, specificity was 61.9%, PPV was 91%, and NPV was 92.8%. There was a significant increase within groups in MDA, procalcitonin, ESR, and calprotectin with *P* value of 0.22, 0.015, 0.000, and 0.009, respectively.

Conclusion

Xpert MTB/RIF as POCT for TB diagnosis is more sensitive and specific than traditional methods of diagnosis using ZN to overcome

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the challenges with weak testing infrastructure especially during the COVID-19 pandemic. Serum calprotectin was significantly increased in the COVID-19 group compared with the TB group C-reactive protein, which was significantly increased in the TB group compared with COVID-19 group.

Keywords: Calprotectin, COVID-19, CRP, D-dimer, LJ, PCT, POCT, pulmonary and extrapulmonary TB, XPERT MTB/RIF, ZN

Introduction

Diseases due to pathogenic mycobacteria cause significant health and economic impacts on humans worldwide. Although mycobacterial diseases primarily affect the lungs, the involvement of extrapulmonary organs has also multiplied, particularly among people with coexisting medical conditions. Besides *mycobacterium tuberculosis* complex organisms, non-tuberculous mycobacteria (NTM) are also known to cause pulmonary and extrapulmonary diseases [1]. Disseminated and primary extrapulmonary mycobacterial infections affect the brain, pericardium, mouth, tongue, lymph nodes of the neck, spine, bones, muscles, skin, pleura, eye, gastrointestinal, peritoneum, and the genitourinary system. Both extrapulmonary mycobacterial diseases of the NTM-infected cases and *M tuberculosis*-infected cases have similar clinical presentation. Moreover, extrapulmonary mycobacterial diseases are complicated by the involvement of diverse bacterial species such as etiological agents. Culture and molecular techniques are used to differentiate NTM from *M. tuberculosis* [2].

The immune status that makes people vulnerable to tuberculosis (TB) may also make them susceptible to coronavirus infection. COVID-19 is already affecting control measures for TB, whereas the possibility of coinfection should be kept in mind [3]. Culture and antimicrobial susceptibility testing are still considered the gold standards for the diagnosis of tuberculosis. Due to a lack of access to mycobacteriology laboratory facilities in many centers, point-of-care tests (POCT) such as the Ziehl–Neelsen (ZN) and Xpert MTB/RIF assay are required for early diagnosis and to prevent the dissemination of drug-resistant strains all over the world [4]. The Xpert MTB/RIF assay (Cepheid, Sunnyvale, California [4], USA) is the most commonly used point-of-care assay for TB that has been endorsed by the WHO [3].

This study aimed to evaluate the usefulness of Xpert MTB/RIF as a POCT for early, rapid, and accurate diagnosis of pulmonary and extrapulmonary TB during the COVID-19 pandemic, its role for the exclusion of nonmycobacterium TB infections, the proportion of patients with active pulmonary TB among COVID-19 patients, and to study the difference in some inflammatory markers between patients with COVID-19, patients with pulmonary TB, and patients infected by both TB and COVID-19.

Figure 1: Acid-fast bacilli slide quality control.

Patients and methods

Patients

This study was conducted between February 2018 and December 2021 (including the peak period of COVID-19 when Chest and Shebin El-Kom Teaching Hospitals turned into isolation hospitals for COVID-19). The study was conducted on 835 suspected TB patients from both hospitals (629 + 206 suspected COVID-19 patients) referred to the Chest Hospital for culture and XPERT MTB/RIF. The number and types of lab procedures [ZN stain, culture on Lowenstein–Jensen (LJ) medium, and XPERT MTB/RIF] used in the diagnosis of all patients are illustrated in Fig. 1.

The purpose and nature of the study were explained to all participants, and their written voluntary consent was obtained before their participation. Approval was taken from the research committee of the general organization of teaching hospitals and institutions (GOTHI) with approval number HSH00035.

Inclusion criteria for enrollment in the study: new cases suffering from chronic cough and hemoptysis, patients with mycobacterial infection relapse, patients for pulmonary lavage, patients who are suspected of having extrapulmonary TB, contacts of patients with multidrug-resistant tuberculosis (MDR-TB), patients not responding to treatment, and HIV-positive cases.

In all, 206 patients were suspected of having TB and COVID-19 during the pandemic and were divided into three groups: group I was positive for TB and COVID-19 ($N = 6$), group II was positive for COVID-19 only ($N = 100$), and group III was positive for pulmonary TB only ($N = 50$) (NB: 50 patients were excluded due to incomplete data).

All of them were subjected to full medical history and clinical evaluation. Routine investigations for COVID-19 patients include C-reactive protein (CRP), D-dimer, ferritin, lactate dehydrogenase (LDH), neutrophils, lymphocytes, neutrophil-to-lymphocyte ratio, and computed tomography (CT) of chest and nasal swab for detection of COVID-19.

Samples were collected for studying inflammatory markers. Blood samples were collected and centrifuged at 3000 rpm for 10 min at 4°C. Serum samples were used for the measurement

of CRP, procalcitonin (PCT), ferritin, and LDH and then the serum was stored at −20°C until the measurements of interleukin-6 (IL-6), malondialdhyde (MDA), and calprotectin. The level of cytokine IL-6 was determined in the serum using enzyme-linked immunosorbent assay (ELISA) kit (Ray Bio Rat IL-6 ELISA kit3607 Parkway Lane, Suite 100 Norcross, GA 30092,USA) according to. MDA level, as a marker of lipid peroxidation, was determined according to the method of Kei, 1978 [5] using Biodiagnostic Company Kits, Egypt. Determination of ferritin and PCT was done according to electrochemiluminescence immunoassay (ECLIA) using Cobas Roche 6000 instrument. Determination of complete blood count, LDH, and D-dimer was done using Roche diagnostic kits by Cobas Integra 400 plus instrument using the Tina-quant technique (Roche Diagnostics Company,Swiss) and lithium heparin samples for D-dimer. Determination of serum calprotectin was done by ELIZA kits using AssayMax Human Calprotectin ELISA Kit (Kit, C. E. Calprotectin ELISA Kit). Another part of blood was taken on EDTA for the determination of CBC, which was done by CELL-DYN Ruby Hematology Analyzer by Abbott (Ruby, Abbott Company).The last part was taken on sodium citrate tubes for erythrocyte sedimentation rate (ESR) test and was done as routine and only the first hour was taken [6].

Nasal swab for confirmation of COVID-19 by PCR.

Samples were collected for microbiological study. The total number of samples (sputum, endotracheal aspirate, cerabrospinal fluid, peritoneal fluid, etc.) were collected according to the site of infection by standard sample collection methods and immediately transported to the microbiology laboratory to be processed in a timely manner. Each sample was made into two parts. One of them is used to prepare ZN acid-fast bacilli (AFB) film. Sputum samples have been decontaminated using *N*-acetyl-l-cysteine-Na OH by the standard decontamination method for the preparation of both ZNAFB film and culture on LJ medium. Controlling staining method and validating positive (pink bacilli) (Fig. 2A) and negative (Fig. 2B) cases with AFB quality control slides Fig. 1 [7].

Samples were cultured on LJ slant tubes which were tested for sterility and performance and then were incubated at 37°C and inspected weekly for 8 weeks before discarding as negative. Mycobacteria were identified by the rate of growth, colony morphology, and by staining with ZN stain. The second tube was used for molecular detection of MTB and its resistance to Rifampicin by the Gene Xpert MTB/RIF assay in Chest Hospital.

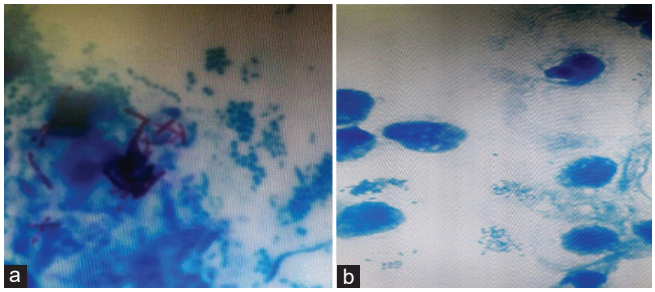

Figure 2: Microscopic positive (A) and negative (B) quality control of AFB QC slide N.B.

NB: only 103 TB patient samples were directly smeared, cultured, and the Xpert MTB/RIF was tested for them. During the spread of the COVID-19 disease, CT scans showed 6 patients with dormant foci of pulmonary TB without their prior knowledge, which was confirmed by the Xpert MTB/RIF. During this period, only Xpert MTB/RIF was done [Table 1], either alone or with a direct smear.

Processing of Gene Xpert MTB/RIF assay (Cepheid)

The Xpert MTB/RIF assay is an automated in-vitro diagnostic test using nested real-time PCR for the simultaneous semiquantitative detection of MTB complex and RIF resistance from raw and concentrated samples by amplifying the MTB-complex-specific sequence of the rpoB gene, which is probed with five molecular beacons (probes A–E) for mutations within the rifampin resistance determining region. According to the manufacturer's instructions, the sample reagent (supplied by the manufacturer) was added to unprocessed samples (2: 1) in falcon tubes, kept at room temperature for 15 min, during which the tubes were twice shaken vigorously 10–20 times or vortexes for at least 10 s. Then 2 ml of the liquefied sample was added to the cartridge of Gene Xpert using a disposable pipette (supplied) after being labeled with sample ID. Then, running on Gene Xpert should be within 30 min of cartridge preparation. The assay time is about 2 h. Results of the assay were interpreted according to manufacturer's instructions and guidelines as either: MTB detected, RIF resistance detected, MTB detected, RIF resistance not detected, and MTB not detected, invalid result. The results were reported as MTB negative or positive and RIF sensitive or resistant [6].

Statistical analysis

In this study, statistical data analysis was carried out using SPSS version 23. Shapiro–Wilks test was used to test normal distribution of variables. Numerical data were expressed as mean ± SD. Qualitative data were summarized as percentages. Correlations between different parameters were done using Spearman's correlation coefficient. The diagnostic sensitivity, specificity, positive predictive (PPV), and negative predictive (NPV) values were calculated. The probability (*P*) values of less than or equal to 0.05 were considered statistically significant.

Table 1: Grading of smear was done according to the following

No. of tubercle bacilli	Grade
None	Negative for AFB
1-9/100 oil immersion field	Scanty
10-99/100 oil immersion field	1+
1-10/1 oil immersion field	2+
>10/1 oil immersion field	3+

AFB, acid-fast bacilli.

Results

In this study, the number of clinical specimens from suspected pulmonary and extrapulmonary TB in both sexes (Table 2) was 835. Samples were collected from a total of 594 participants males (71.13%) and 241 (28.87%) females. Patients with suspected pulmonary TB (623) and different clinical samples (212) from patients suspected of extra pulmonary TB are shown in Fig. 3. The age of the study participants ranged from 18 to 78 years, with a mean age of 41.91 ± 16.2 years.

All 835 samples (pulmonary and extrapulmonary samples) were tested by Gene Xpert MTB/RIF. All specimen results were obtained within 2 hours of starting the analysis (26 specimens were invalid).

Out of 835 samples only 441 of them were tested by ZN only.

Sensitivity and specificity of POCT (ZN and XPERT MTB\RIF) in comparison to culturing on LJ medium (Table 3). We prepared 103 samples (78 samples were obtained from males and 25 from females, with a male to female ratio of 3.1: 1) to be tested by the three methods (gene Xpert MTB/RIF, ZN staining, and culture on LJ media). In all, 82/103 (79.6%) patients had been microbiologically confirmed with mycobacterium TB detected in their culture. When the samples were examined by smear microscopy, 64/103 (62%) were smear-positive and culture-positive TB (S+/C+), whereas 18/103 (17%) were smear-negative and culture-positive TB (S-/C+). Twenty-one patients out of 103 (20%) were shown to have no TB (C-). These patients were smear-negative and culture-negative.

Concerning that confirmed positive culture was used as the gold standard, the patients were divided into two categories:

Microbiologically confirmed TB (S+/C+)=82, (S−/C+)=18.

Category 2: No microbiological proof of TB no TB (C−)=21.

Compared with culture, sensitivity, specificity, PPV, and NPV for ZN smear were 77.1, 100, 100, and 53.8%, respectively, as shown in Table 3.

Performance of XPERT MTB/RIF assay:

From the 103 samples examined, 89 (86%) were positive and 14 (14%) were negative. Eight false-positive results were recorded in the results of the XPERT MTB/RIF assay. Compared with the culture, the sensitivity was 98.8%, specificity was 61.9%, PPV was 91%, and the NPV was 92.8%, as shown in Table 3.

Eight samples were tested positive by XPERT MTB/RIF but negative by culture. These samples were from patients who presented with cough, dyspnea, and chest pain. CT

Table 2: Number of clinical specimens from suspected pulmonary and extrapulmonary TB in both sexes

Specimens	Total no	Male, *n* (%)	Female, *n* (%)	Age (mean±SD)
BAL	27	19 (70.3)	8 (29.7)	
Sputum	596	423 (70.9)	173 (29.1)	
Peritoneal fluid	30	22 (73)	8 (27)	
Pleural Eff	52	37 (71.1)	15 (28.9)	
CSF	98	74 (75.5)	24 (24.5)	
Others	32	19 (59.4)	13 (40.6)	
Total pulmonary	623	442 (70.9)	181 (29.1)	
Total extrapulmonary	212	152 (71.7)	60 (28.3)	
Total (100%)	835	594 (71.13)	241 (28.87)	41.91±16.2

Others sites, for example, renal, lymph node, bone. CSF, cerebrospinal fluid; TB, tuberculosis.

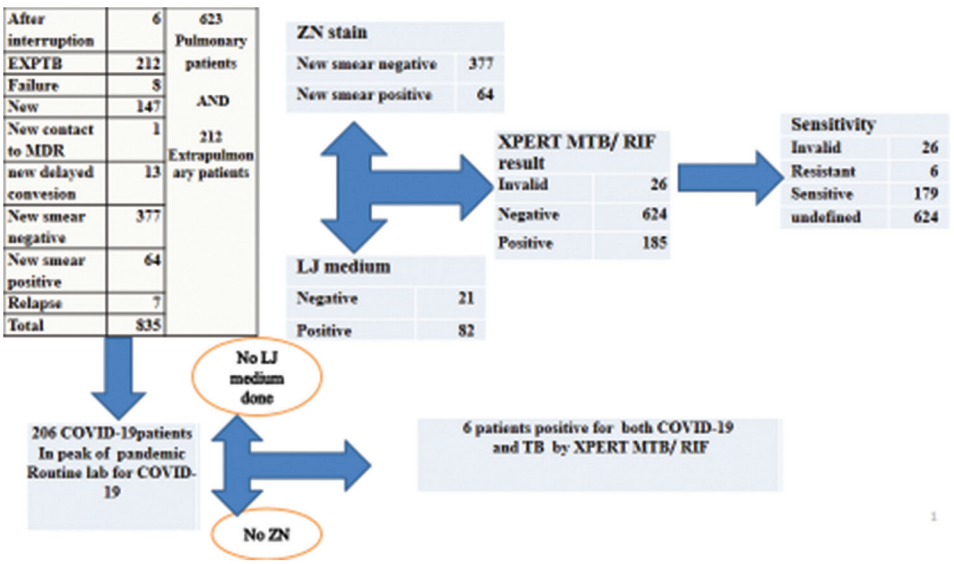

Figure 3: The number and types of lab procedures (Ziehl–Neelsen stain, culture on Lowenstein–Jensen medium, and XPERT MTB/RIF) used in the diagnosis of all patients.

scan revealed the presence of pulmonary consolidation and cavitation and anti-TB drugs was initiated.

One sample was tested negative by XPERT MTB/RIF but positive by culture. This sample was non-MTB.

We found that all samples with a smear examination positive had a positive XPERT MTB/RIF test, thus corresponding to the overall sensitivity of the XPERT MTB/RIF to detect smear-positive samples of 100%.

Out of 89 specimens with an MTB-positive XPERT test, rifampicin resistance was detected in six cases (6.7%). All of them were cultured and smear-positive.

For studying the difference of some inflammatory markers (Table 3) between patients with COVID-19, patients with pulmonary TB, and patients infected by both TB and COVID-19, 206 patients were suspected to have comorbid TB and COVID-19 during the pandemic. They were divided into three groups: group I positive for TB and COVID-19 ($N = 6$), group II positive for COVID-19 only ($N = 100$), and group III were positive pulmonary TB only ($N = 50$) (NB: 50 patients were excluded due to incomplete data).

There was a significant difference in CRP, D-dimmer, and lymphocytes within groups ($P = 0.040$, 0.007, and 0.028, respectively) and no significance in others. There was a significant increase in CRP and lymphocytes between groups 2 and 3 ($P = 0.027$ and 0.007, respectively). D-dimer increased significantly between groups 2 and 3 ($P = 0.004$) and significantly between G1 and G2 ($P = 0.015$). There was a significant increase within groups in PCT, ESR, MDA, and calprotectin with P values of 0.015, 0.000, 0.22, and 0.009, respectively. There was a significant increase in PCT results for G3 compared with G1 ($P = 0.022$). There was a significant increase in ESR results of G1 compared with G2 and in G3 compared with G2 ($P = 0.004$ and 0.000, respectively). There was no significance between G1 and G3 within the ESR results. Results of MDA and calprotectin showed a significant increase in G2 compared with G3 with P values of 0.033 and 0.045, respectively and no significance between G1 and G2, but there is a significant increase in results of calprotectin in G1 compared with G3 with a P value of 0.019.

No significance in results of IL-6 within groups Table 4.

Discussion

During the pandemic, COVID-19 was the main concern in every clinic and as there were overlapping respiratory diseases which may result in delaying the diagnosis and treatment [7]. Early diagnosis of TB is important for patient management and successful outcomes [8]. Sputum smear microscopy is one of the most effective tools for identifying people with infectious TB. Smear-positive patients are up to 10 times more infectious than smear-negative patients. It is still the primary method for the diagnosis of TB in low-income and middle-income countries, which are the only cost-effective tool for diagnosing infectious patients monitoring their progress in treatment and confirming cure. However, sensitivity values are low, with limited specificity that cannot differentiate between MTB and NTM, multiple visits, seldom on the same day [9].

Culture and antimicrobial susceptibility testing are still considered the gold standards for the diagnosis of TB. Due to the lack of access to bacteriology laboratory facilities in many centers, POCTs are required for early diagnosis and to prevent the dissemination of drug resistance strains all over the world [3]. The Xpert MTB/RIF assay (Cepheid) is the most widely used point-of-care assay for TB that has been endorsed by the WHO (allowing for a quick treatment turnaround time of a few minutes or hours (in a single clinical encounter), avoiding patient loss to follow-up [10].

This study aimed to evaluate the usefulness of Xpert MTB/RIF as a POCT for early, rapid, and accurate diagnosis of pulmonary and extrapulmonary TB during the pandemic of COVID-19, its role for exclusion of nonmycobacterium TB infections, the proportion of patients with active pulmonary TB among COVID-19 patients, and to study the difference in some inflammatory markers between patients with COVID-19, patients with pulmonary TB, and patients infected by both TB and COVID-19.

In this study, the male-to-female sample ratio was 71.7%–28.3% (2.5: 1). Their age ranged from 18 to 78 years, with a mean age of 41.91 ± 16.2 years. In the study carried out by Ganguly *et al*. [9], male participants accounted for 85.71% of

Table 3: Sensitivity and specificity of POCT (ZN and XPERT MTB/RIF) in comparison to culturing on LJ medium (gold standard)

Total no=103	LJ medium, *n* (%)		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
	Positive (no.=82)	Negative (no.=21)					
ZN							
Positive (no.=64)	64 (77.1)	0	77.1	100	100	53.8	82.5
Negative (no.=39)	18 (21.9)	21 (100)					
XPERT MTB/RIF							
Positive (no.=89)	81 (98.8)	8 (38.1)	98.8	61.9	91	92.8	91.3%
Negative (no.=14)	1 (1.2)	13 (61.9)					

ZN true positive=64, true negative=21, false-positive=0, false negative=18. XPERT MTB\RIF true positive=81, true negative=13, false-positive=8, false negative=1. LJ, Lowenstein-Jensen; NPV, negative predictive value; POCT, point-of-care test; PPV, positive predictive value; ZN, Ziehl-Neelsen.

Table 4: Difference in inflammatory markers between the studied groups (COVID-19, TB, and both)

Group	Neutrophil	Lymph	Neutrophil/ lymph	Procalcitonin (ng/ml)	ESR]	IL-6 (pg/ml)	Ferritin (ng/ml)	CRP (mg/dl)	D-dimer (µg/ml)	LDH (IU/L)	MDA (µmol/L)	Calprotectin (µg/dl)
1												
Mean	9.68	0.46	21.9	0.135	61.17	26.78	225.33	55.32	2.14	375.50	2.53	13.71
N	6	6	6	6	6	6	6	6	6	6	6	6
SD	4.89	0.24	20.03	0.179	24.47	7.9	87.143	60.5	1.09	153.384	0.28	15.8
2												
Mean	9.00	1.29	13.98	0.49	31.27	26.9	123.82	65.8	3.74	348.91	3.75	3.56
N	100	100	100	100	100	100	100	100	100	100	100	100
SD	3.08	0.82	14.31	0.304	12.92	7.14	123.32	36.2	1.166	144.10	1.44	3.08
3												
Mean	10.08	0.96	28.91	0.919	74.27	25.72	202.20	118.72	1.517	354.00	2.7	1.53
N	50	50	50	50	50	50	50	50	50	50	50	50
SD	3.475	0.78	21.44	0.71	23.917	8.21	107.898	62.67	1.90	166.461	0.47	0. 67
ANOVA between groups	0.795	0.028*	0.201	0.015*	0.000*	0.934	0.145	0.040*	0.007*	0.942	0.022*	0.009*
ANOVA between G1 and G2	0.731	0.444	0.357	0.20	0.004*	0.976	0.096	0.658	0.015*	0.727	0.06	0.052
ANOVA between G1 and G3	0.848	0.074	0.528	0.022*	0.301	0.804	0.664	0.067	0.478	0.801	0.426	0.019*
ANOVA between G2 and G3	0.459	0.007*	0.074	0.086	0.000*	0.729	0.139	0.027*	0.004*	0.941	0.033*	0.045*

ANOVA, analysis of variance; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL-6, interleukin-16; LDH, lactate dehydrogenase; MDA, malondialdehyde; TB, tuberculosis. *Significant≤0.05.

the total, compared with 14.29% for females. This may be due to the fact that male participants were more exposed to risk factors for TB than females.

In this study, 79.6% (82/103) of patients had their cultures positive for TB. ZN smear microscopy diagnosed 62% (64/103) of cases, while Xpert MTB/RIF detected 86% (89/103) of cases.

Considering conventional culture as a gold standard method, the sensitivity of Xpert MTB/RIF was 98.8%, which is comparable to the study by Willamson *et al*. [11] and Carriquiry *et al*. [12], who reported a sensitivity of 96.7 and 100% in Pero and New Zealand, respectively.

However, reports indicate that the Xpert MTB/RIF test sensitivity is as low as 62.6% in South Africa and 67.6% in Adama, Ethiopia [13,14].

The higher sensitivity detected in the present study may be explained by the use of samples under more selective conditions than in other studies, which used samples from consecutive patients without any previous selection and the use of frozen samples, which may cause some degradation of the TB DNA.

The specificity of the Xpert MTB/RIF assay was 61.9%. Higher specificity was found by other studies [14,15], which have shown a specificity ranging from 94.1% to 100%. This difference may be explained by the presence of eight false-positive results detected by Xpert MTB/RIF from eight patients whose sputum was cultured negative. These positive results may be true positives due to the high sensitivity of the assay, the presence of residual DNA of old dead organisms in patients with a previous history of TB, or a subclinical relapse of the disease. This agrees with García-Basteiro *et al*. [4], who reported that patients with previous TB, who are an epidemiologically important subpopulation who have presented with symptoms, have old mycobacterial genomic DNA in their lungs that can cause false-positive results (for active TB). Thus, as diagnostic tests improve in sensitivity, specificity is likely to be compromised in patients with a history of TB unless special precautions are taken.

In this study, we found that the sensitivity and specificity of the Xpert MTB/RIF assay were higher than those of ZN smear microscopy. These results are in harmony with most of the studies conducted previously for the evaluation of the performance of Xpert MTB/RIF [16,17]. In addition, the sensitivity of ZN smear may vary between different laboratories, which do not occur with nucleic acid-based assay methods.

ZN smear microscopy is one of the most effective tools for identifying people with infectious TB, monitoring their progress in the treatment, and confirming cures. Smear-positive patients are up to 10 times more infectious than smear-negative patients. The threshold of detection of AFB in the sputum is 10^4–10^5 CFU/mL. Technically, smear microscopy is

inexpensive, easy to perform, and highly specific in areas with high prevalence. However, sensitivity values are low. Under optimal conditions, maximum sensitivity has been found to be up to 60% higher under optimal conditions when compared with that of cultures [4].

Comparing the results of the Xpert test with those of smear microscopy using the international smear grading system, we found that all cases with negative results by Xpert MTB/RIF were also negative by smear examination.

Out of 89 specimens with an MTB-positive result by XPERT, six rifampicin-resistant samples were detected (6.7%). The six rifampicin-resistant samples were culture-positive and smear-positive. This agrees with Khalil and Butt [17], who found that 6 out of 93 isolates (6.5%) showed rifampicin resistance, while 87 isolates (93.5%) were susceptible strains.

In our study, 6 (2.9%) out of 206 COVID-19 patients had pulmonary TB while Aggarwal *et al*., [18] reported that 0.99% of the COVID-19 patients had active pulmonary TB, which explained that the lower TB proportion is due to under-recognition or underreporting of active TB among COVID-19 patients or due to protection strategies commonly employed by people with respiratory disorders, is not certain.

Both diseases are primarily respiratory illnesses, eliciting a hyperinflammatory state in the lung. The hyperinflammatory background induced by COVID-19 could hurry TB disease progression and vice versa. Moreover, the hyperinflammatory conditions associated with COVID-19 could favor *M. tuberculosis* reactivation. These concerns are further emphasized by many large epidemiological studies showing that an increased hazard of COVID-19-related death is independently associated with active TB [19].

There was a significant difference in CRP, D-dimer, and lymphocytes within groups (P = 0.040, 0.007, and 0.028, respectively, and no significance in others). There was a significant increase in CRP and lymphocytes between groups 2 and 3 (P = 0.027 and 0.007, respectively). The D-dimer showed a highly significant increase between groups 2 and 3 (P = 0.004) and a significant increase between G1 and G2 (P = 0.015). There was a significant increase within groups in PCT, ESR, MDA, and calprotectin with P values of 0.015, 0.000, 0.22, and 0.009, respectively. There was a significant increase in PCT results for G3 compared with G1 (P = 0.022). There was a significant increase in ESR results of G1 compared with G2 and in G3 compared with G2 (P = 0.004 and 0.000, respectively). There was no significance between G1 and G3 within the ESR results. Results of MDA and calprotectin showed a significant increase in G2 compared with G3 with P values of 0.033 and 0.045, respectively and no significance between G1 and G2, but there was a significant increase in the results of calprotectin in G1 compared with G3 with a P value of 0.019.

No significance in the results of IL-6 within groups. Hashem *et al*. [20] reported that lymphopenia and high neutrophil counts are simple initial parameters proposed to directly distinguish between non-severe and severe COVID-19 patients. In severe COVID-19 patients, increased D-dimer values may also be indicators of a worse prognosis, which is clarified by deregulated coagulopathy. Inflammation-related proteins seem to also provide valuable prognostic data. Elevated PCT, CRP levels, and serum ferritin distinguish between mild and severe COVID-19 cases. Other inflammatory cytokines such as IL-6 and biochemical factors including lactic dehydrogenase (LDH) may also be markedly altered in severe COVID-19 patients. All of these results were in accordance with the results of this study.

Ding *et al*. [21] reported an increase in PCT, ESR, and CRP in patients with pulmonary TB when they were studying the effect of linezolid on serum PCT, ESR, and CRP in patients with pulmonary TB and pneumonia.

The results of calprotectin are in accordance with Mahler *et al*. [22], as they used blood calprotectin as a biomarker of COVID-19 severity. In addition Larsson *et al.* [23] reported significantly elevated fecal calprotectin, serum calprotectin, and CRP levels compared with the control in pulmonary TB groups.

The results of CRP are in accordance with the results of Ciccacci *et al*. [24] as they showed that plasma levels of CRP were significantly higher in TB-positive compared with TB-negative participants.

Also Cudahy *et al.* [25] reported that elevated D-dimer has been associated with pulmonary TB and HIV/TB coinfection, and may presage death from pulmonary thromboembolic disease. Danwang *et al.*, [26] reported that elevated D-dimer signifies a hyperfibrinolytic state and an increased inflammatory burden induced by SARS-CoV-2 infection.

The present study supports the view that there may be a link between lipid peroxidation and cytokine response and relative roles of cytokines and lipid peroxidation in the pathogenesis of TB and COVID-19 patients. This is indicated by the results of MDA, as it indicates lipid peroxidation due to inflammation. This is in accordance with the study by Kulkarni [27], as they reported a significant increase in MDA in pulmonary TB patients compared with controls.

Conclusion

The Xpert MTB/RIF as POCT for TB diagnosis is more sensitive and specific than traditional methods of diagnosis using ZN to overcome the challenges of weak testing infrastructure, especially during the COVID-19 pandemic.

Serum calprotectin was significantly increased in the COVID-19 group compared with the TB group as the CRP was significantly increased in the TB group compared with the COVID-19 group.

Recommendation

A policy of bidirectional screening of both pulmonary TB and COVID-19 patients is suggested to be implemented, especially in nations with a high TB burden.

Ethical Clearance

Ethics committee approval was taken from the research committee of the General Organization of Teaching Hospitals and Institutions (GOTHI) with approval number HSH00035.

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Conflicts of interest

There are no conflicts of interest.

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