World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.008

COMPARATIVE STUDY BETWEEN GENEXPERT MTB/RIF ASSAY AND DIRECT SMEAR MICROSCOPY(DSM) FOR DETECTION OF MYCOBACTERIUM TUBERCULOSIS BACIILI IN SMEAR NEGATIVE SUSPECTED PULMONARY TUBERCULOSIS AT BAGHDAD ALRUSAFA RESPIRATORY CENTER

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Article Received on 21/11/2018 **Article Revised on 12/12/2018** **Article Accepted on 02/01/2019**

ABSTRACT

For the diagnosis of PTB the detection of Acid Fast Bacilli (AFB) in expectorated sputum is still crucial, especially in developing countries including Iraq, Introduction of GeneXpert assay has created a major breakthrough for diagnosis of tuberculosis. Providing a rapid and accurate way of identifying TB patients in high TB-burden, low-income countries. Several patient factors may influence diagnostic performance of direct smear, however GeneXpert has increased the trend of diagnosted patients in Alrusafa Respiratory Center? When AFB is detected in sputum, the diagnosis of PTB is certain.

KEYWORDS: GeneXpert, Acid Fast Bacilli (AFB), PTB.

BACKGROUND

TB is an infectious disease caused by the bacillus Mycobacterium tuberculosis. It typically affects the lungs (pulmonary TB) but it can also affect other sites (extrapulmonary TB). TB is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS. Iraq is considered to be a middle burden country with TB, and occupies rank 108 globally and 7 in eastern Mediterranean region among countries with TB burden size. According to WHO report, the estimated incidence of TB in Iraq is 43/100000 population. The mortality is 3/100000. Iraq's NTP recorded a total of 7,246 TB cases in 2016 alone.[1]

In 2016, there were an estimated 1.3 million TB deaths among HIV-negative people. In 2016, there were 600 000 new cases with resistance to rifampicin (RRTB), the most effective first-line drug, of which 490 000 had multidrug-resistant TB (MDR-TB.[2] Most deaths from TB could be prevented with early diagnosis and appropriate treatment. Millions of people are diagnosed and successfully treated for TB each year, averting millions of deaths (53 million 2000–2016), but there are still large gaps in detection and treatment.[3]

However diagnostic problem started when patients with suspected PTB have a negative sputum smear. It has always been recognized that a proportion of patients are sputum smear negative using the Ziehl-Nelsein (ZN) stain (the commonly used stain in most laboratories of the TB Centers in Iraq to detect AFB in sputum). This is a simple, rapid and cheap test but lacks sensitivity of a sputum test.[4] About 5000 bacilli per milliliter of sputum must be present for it to be positive. Chest radiography is not always helpful in smear negative patients. The radiographic distinction between active and inactive tuberculosis can be difficult.[5] In fact, substantial numbers of patients are treated for tuberculosis without definitive diagnostic criteria. The Iraqi National AntiTB Program(NTP) uses a smear negative PTB diagnostic algorithm adopted from the World Health Organization (WHO)[5] (Figure 1). Patients who came to the clinic with symptoms suggestive of PTB had their sputum examined. Smears were considered positive if AFBs were seen on smear from any of the two sputum samples. Patients found to have sputum smear positive were treated for tuberculosis according to the Iraqi National Tuberculosis program treatment guidelines. Those who were two times smear negative and chest x-ray results were abnormal, were enrolled into the study and consequently asked to bring one more sputum sample which was sent to the Laboratory at Al rusafa Respiratory Center for GeneXpert which is the only rapid test for diagnosis of TB. It can provide results within 2 hours. It was initially recommended by WHO (in 2010) for diagnosis of pulmonary TB in adults. Since 2013, it has also been recommended for use in children and to diagnose specific forms of extrapulmonary TB.

The test has much better accuracy than sputum smear microscopy; Sputum smear microscopy –Developed more than 100 years ago, this technique requires the examination of sputum samples using a microscope to determine the presence of bacteria. In the current case definitions recommended by WHO, one positive result is required for a diagnosis of smear-positive pulmonary TB; Culture-based methods – The current reference standard, they require more developed laboratory capacity and can take up to 12 weeks to provide result. Globally, use of rapid molecular tests is increasing, and many countries are phasing out the use of smear microscopy for diagnostic purposes (although microscopy and culture remain necessary for treatment monitoring). Despite advances in diagnostics, a considerable proportion of the TB cases reported to WHO are still clinically diagnosed rather than bacteriologically confirmed. In 2016, for example, only 57% of the pulmonary cases reported to WHO were bacteriologically confirmed. Reasons for a low proportion of cases being bacteriologically confirmed should be assessed at country level, as should reductions over time. The microbiological detection of TB allows patients to be correctly diagnosed and started on the most effective treatment regimen as early as possible, and is critical for infection control. Most clinical features of TB and abnormalities on chest radiography or histology results generally associated with TB have low specificity, which may lead to false diagnoses of TB, and hence to people being enrolled in TB treatment unnecessarily.[1] Gene Xpert (Omni) – were expected to be available for WHO evaluation in 2017. Omni is described by the manufacturer as a small, light and durable instrument designed for simultaneous testing for TB and rifampicin-resistant TB using either Xpert® MTB/RIF cartridges or the next-generation Xpert MTB/RIF Ultra cartridges. It is aimed at facilitating wider access to rapid molecular testing for TB and rifampicin resistance in decentralized settings, aided by specific features such as built-in batteries and use of cartridges that incorporate a near-field communication chip to allow cellular transfer of data. The Ultra cartridge showed significantly better performance (increased sensitivity) than the current Xpert MTB/RIF cartridge in detecting M. tuberculosis in specimens with low numbers of bacilli. This was particularly the case for smear-negative, extrapulmonary specimens (notably cerebrospinal fluid) and specimens from children. WHO recommendations for the use of Xpert MTB/RIF as the initial diagnostic test for all adults and children with signs and symptoms of TB, and for the testing of selected extra pulmonary specimens (cerebrospinal fluid, lymph nodes and tissue specimens) now also apply to the Ultra assay.[3]

Cost affordability and Cost-effectiveness Analyses

The cost of achieving the diagnostic targets in the Global Plan to Stop TB, (2011–2015) with and without use of Xpert MTB/RIF was appraised for three population groups, *i.e.* TB patients considered at risk of having MDR-TB, people living with HIV with TB signs and symptoms, and all people with TB signs and symptoms. The price at the end of 2010 was US$16.86 per cartridge. There were four main findings. First, a diagnostic strategy using Xpert MTB/RIF with follow-on DST for rifampicin-positive cases was a lower cost approach for reaching the 2015 targets for diagnosis of MDR-TB, both globally and in all high TB and high MDR-burden countries, compared with reliance on conventional culture and DST only. Secondly, using Xpert MTB/RIF to diagnose TB in people living with HIV in high HIV-prevalence settings was of similar or lower cost, compared with the conventional diagnostic algorithm (based on culture and radiography) recommended by WHO, in most countries. Thirdly, the total cost of using Xpert MTB/RIF to diagnose MDR-TB and TB among people living with HIV was a small fraction (<5%) of total spending on TB control in 2010. Finally, the cost of using Xpert MTB/RIF to test all people with TB signs and symptoms was much higher compared with conventional diagnosis based on smear microscopy and radiographs, but in middle-income countries would be relatively affordable compared with total spending on TB care and control.[6]

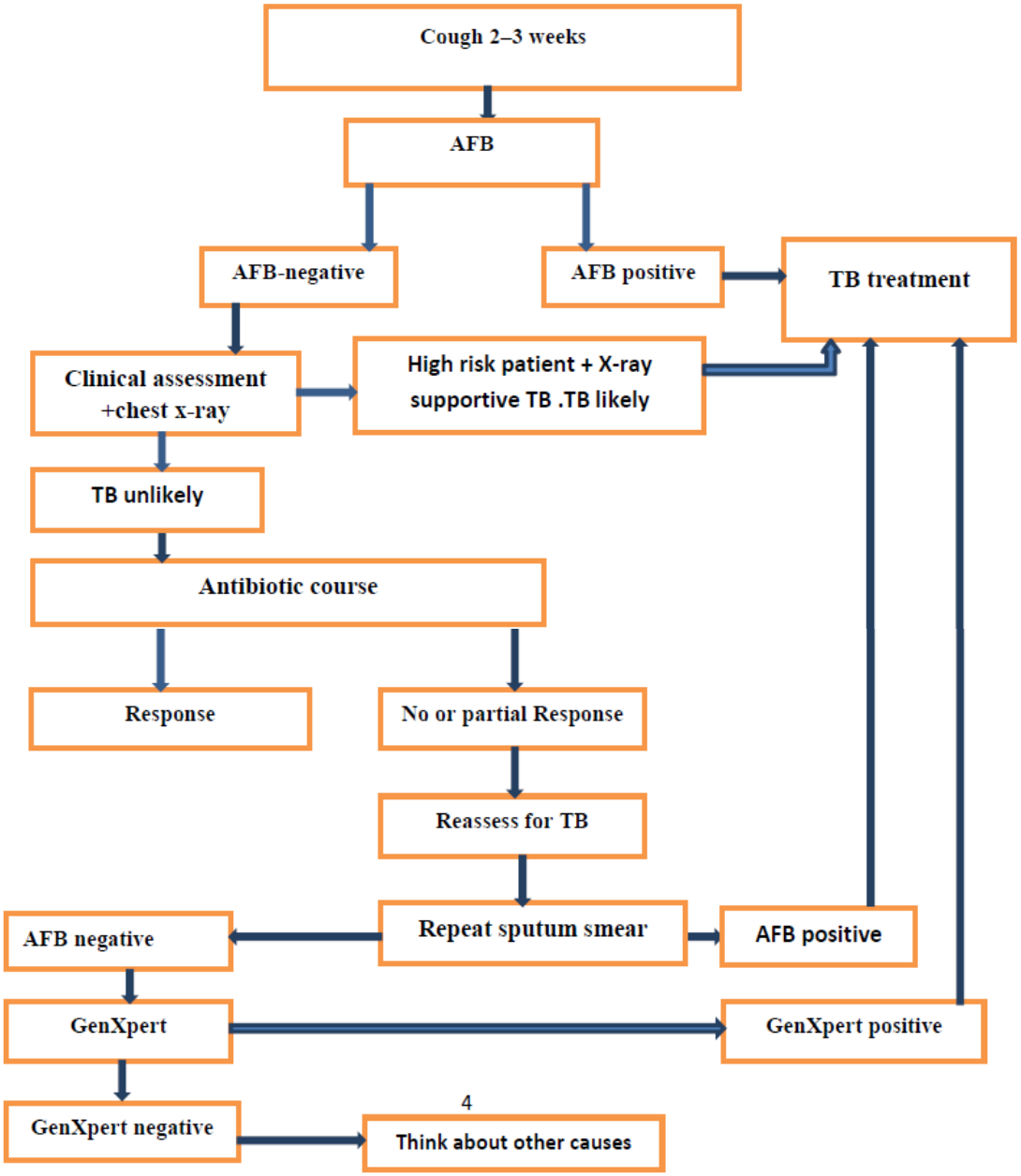


Figure 1: Smear negative PTB diagnostic algorithm.

OBJECTIVES OF THIS STUDY

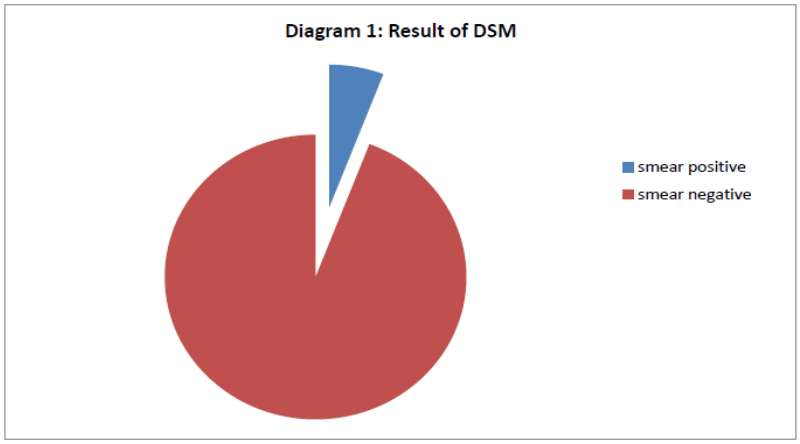
Diagnosis of smear negative pulmonary tuberculosis is made by an algorithm recommended by the National Tuberculosis Program(NTP) that uses symptoms, signs and laboratory results. The objective of this study is to determine the sensitivity and specificity of the Genexpert used for the diagnosis of sputum smear negative pulmonary tuberculosis in Baghdad Al rusafa Respiratory Center.

METHODS

All suspected patients who had chest X-rays abnormality with sputum smear negative were enrolled in study. They were sent for GenXpert.

RESULTS

During the study, 412 patients were enrolled from May 2017 to May 2018 (Table 1). all of them had chest X-rays (,61 Of those had chest X-rays abnormality). 388 (94.17%) were AFB negative, 24(5.82%) were AFB positive (37 of 61 had had significant chest X-rays findings but they were smear negatives).3 patients had CA lung and 4 had pleural effusion confirmed by CT. The remaining 30 were sent for GenXpert of whom 28 (93.33%) were positive and 2 (6.66%) negatives respectively (diagram 1).



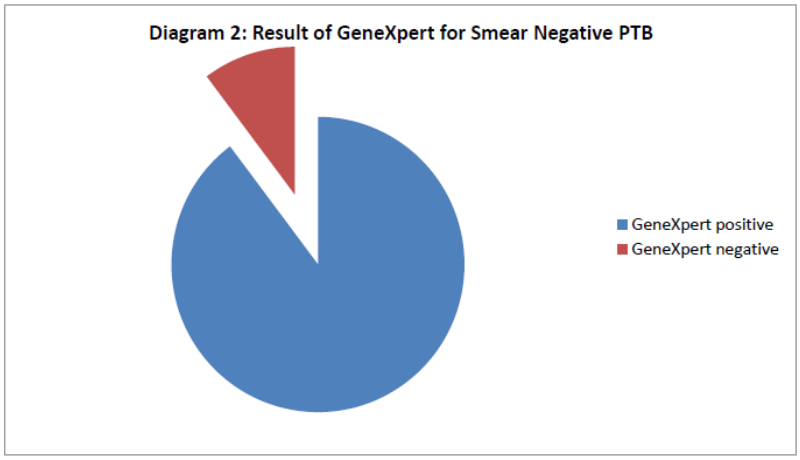


Table 1: Diagnostic direct smearplus chest x-rays.

	AFB	Abnormal CXR	Normal CXR
Smear positive?	24	24	0
Smear negative	388	37	351
Total	412	61	351

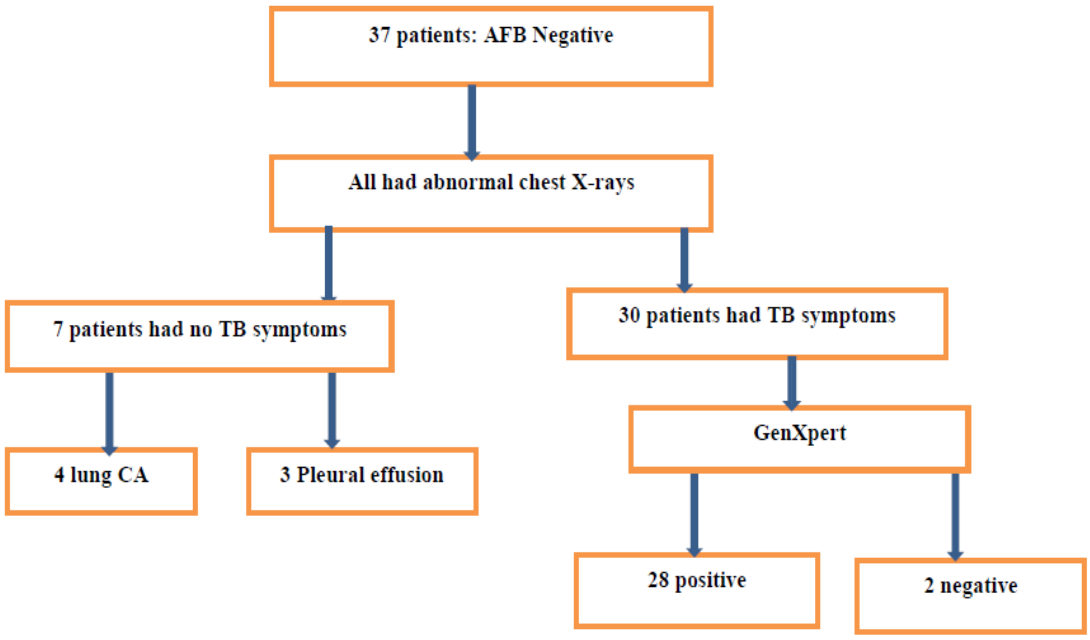


Figure 2: GeneXpert for Smear negative patients who had chest x-ray findings.

DISCUSSION

This study shows that patients who had a negative result for AFB and abnormal chest X-rays plus signs and symptoms of PTB, a significant proportion had sputum GenXpert positive result for MTB (93.33%) and (6.66%) was negative. Therefore our data indicates that smears did not detect PTB in a very large proportion of smear negative patients. GenXpert being a good standard for the diagnosis of Tuberculosis disease[5,12] and sputum smear is not a highly sensitive tool in the diagnosis of PTB. One of the reasons for low sensitivity is reported to be due to the fact that 10^4/ml are required for AFB to be seen using smear microscopy.[6,7] Although the gold standard for the diagnosis of Tuberculosis involves the isolation and identification of Mycobacterium Tuberculosis (MTB) using culture or GenXpert but the culture take long time and Genxpert take only two hours.[8] The cost of doing GenXpert are prohibitive in most developing countries but it is affordable in Iraq. Sputum smear microscopy remains the main diagnostic tool for PTB that allows initiation of treatment and monitoring of patient progress. As sputum smear and microscopy is not a very sensitive tool in the diagnosis of PTB, presumptive diagnosis is usually made based on an algorithm of clinical and radiological criteria. This is commonly termed as AFB negative PTB. In some cases when sputum smears are negative but the patient has clinical features highly suggestive of PTB. The current diagnostic algorithm leading to the establishment of the diagnosis of AFB smear negative PTB is inefficient; it over-diagnoses PTB and misses a lot of people with active disease.[9] Introduction of GeneXpert MTB/RIF assay has constituted a major breakthrough for smear negative tuberculosis (TB) diagnostics which is more sensitive diagnostic tool will prevent the unnecessary cost of treating individuals who do not have TB and at the same time it will prevent the further spread of TB. This emphasizes the need of GenXpert in all TB centers and the need of further research in order to identify a better diagnostic tool for a smear negative PTB.

CONCLUSION

The current practices of establishing pulmonary tuberculosis diagnosis are not sensitive and specific enough to establish the diagnosis of Acid Fast Bacilli smear negative patients. This study is an attempt to improve on the diagnostic algorithm in our center, the study looked at the clinical presentation of the patients to identify clinical laboratory and radiological features that are associated with smear negative patients who had signs and symptoms suggestive of PTB and which can be confirmed by GenXpert. This is the best and the fast way for diagnosis of suspected pulmonary tuberculosis. The sensitivity of GeneXpert is much higher than AFB smear microscopy in respiratory samples (sputum). It can be a useful diagnostic method in suspected pulmonary tuberculosis who are smear negative. Furthermore, it is useful tool to detect Rifampicin resistance.

RECOMMENDATIONS

1. Our data indicates that DSM did not detect PTB in a very large proportion of smear negative patients. GenXpert being a good standard for the diagnosis of Tuberculosis disease.
2. It is recommended to introduce GenXpert in all respiratory centers to be able to identify actually smear negative PTB.
3. Results of GenXpert tests should be communicated to the clinician without waiting for culture.

List of Abbreviations

Anti-TB: Anti-tuberculosis drugs.
WHO: World Health Organization.
PTB: Pulmonary Tuberculosis.
AFB: Acid Fast bacilli.
MTB: Mycobacterium Tuberculosis Mycobacterium Tuberculosis.
NTP: National Tuberculosis Program.
ZN: Ziehl-Nelseen.
TB: Tuberculosis.
DSM: Direct smear microscopy.

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