Molecular Detection of pulmonary Tuberculosis using the Gene X pert MTB/RIF assay in Kurdistan of Iraq

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ABSTRACT

Tuberculosis has been potentially diagnosed by an innovative Gene Xpert Mycobacterium tuberculosis rifampcin tool substantially impacted the quick detection of RIF resistance in clinical samples. Multi drug-resistant tuberculosis signify the remarkable resistance to the conventional (isoniazid, rifampicin against tuberculosis.

The gene x pert MTB/RIF has been clinically and microscopically utilized for the detection of the multivariate pulmonary specimens of early pulmonary tuberculosis cases. Robust screening study had been implemented at Erbil community in the chest and respiratory diseases centre in the period June 2017- June 2018.

Two hundred fifty sputum samples were obtained from TB suspects. All samples were tested on Gene Xpert for MTB/RIF detection after acid fast bacilli microscopy. 75 (30%) sputum samples were AFB smear positive and 175 (70%) were negative. In MTB/RIF assay 85 (34%) were MTB positive and 165 (66%) were negative. The MTB/RIF assay also detected 7 RIF-resistant specimen and 78 RIF-susceptible specimens, and the results were confirmed by drug susceptibility testing. Sensitivity and specificity of Gene xpert were 100% and 94.29% respectively, compared with the conventional method.

Hence from the overall conclusion, we can acknowledge that the MTB/RIF assay has simplified and solve the dramatic challenge of multiple TB cases and cut short with positive impact the dilemma of empirical TB treatment drugs.

1. INTRODUCTION

WHO has launched a new Cepheid Gene Xpert diagnostic modality for Mycobacterium tuberculosis and rifampcin resistant (Daum et al., 2016).

This expedited identification of MTB/RIF resistant has a great impact on disease management overall owing to the easily and accessible disease transmission among the population (Arzu et al., 2011).

WHO,(2014) recorded about 9000000 people infected with TB in (2013), also the main threat of MDR/TB was roughly approximated to be 480000 patient posing 210000 cases-fatalities. (Keira et al., 2016).

In order to supplant the conventional low diagnostic yields and modest sensitivity, the newer modalities of nucleic acid tests have been evolved, the latter method has gained a high sensitivity for AFB positive specimen versus lower acid-fast bacilli negative
specimen. (WHO 2010). (Panayiotis et al., 2011).

Great diagnostic burden still sways on the issues related to the poor gain of smear microscopy and time-consuming culture and also to the labile sensitivities of molecular types. (Giulia et al., 2017; Lewinsohn et al., 2017).

The poor prognosis and down-running outcome are directly proportional to the ill-diagnosed lab. Moreover, microbiological settings, leading to the delayed clinician response in the wake of disease management, hence posing ill-treated patients with the emergence of widespread resistance.

The key core organ that plays a pivotal disease contraction for T.B. infection is the lung whether a new or relapsed cases are ranging between 54 to 97 % of the overall reported cases. (WHO 2017).

Delayed or faulty T.B. diagnosis has promoted disease transmission among the population, the main and critical strategy is to reduce or prevent the eruption of epidemic cases and to enhance rapid and precise diagnosis also identify and notify for premature T.B. cases through broadened and proper screening programs.

The conventional smear microscopy and culture methods have gained poor or modest sensitivities in addition to other limitations concerning the delayed timeframe and ill-equipped skeleton of the lab. Facilities in some countries leading to attenuated access for proper diagnosis and treatment. (Detjen et al., 2015; Eutkemeyer et al., 2016)

2. MATERIALS AND METHODS

At the Chest and Respiratory Diseases Center in Erbil city a statistical study employed between the time span June 2017_June 2018. Those patients who suffer of clinical and radiological (x-ray) were included in the study by obtaining sputum samples and sending for AFB staining and x pert MTB/RIF test.

2.1 Smear microscopy

All specimens were stained for acid-fast microscopic examination using Ziehl-Neellesen before sample concentration. The grade of acid-fast bacilli positivity was assigned to one of the four categories (1+, 2+, 3+, 4+) as per Clinical and Laboratory Standards Institute (CLSI) guidelines CLSI Publishes New Microbiology Guideline, M48—Laboratory Detection and Identification of Mycobacteria in (2018)

2.2 X pert MTB/RIF assay

Early morning coughing in the appropriate sterile container is paramount for our laboratory Test and study.

The assay utilises single-use plastic cartridges with multiple chambers that are preloaded with liquid buffers and lyophilized reagent beads necessary for sample processing, DNA extraction and hemi nested rt-PCR. (Zhang et al., 2016, Gayen et al., 2016).

The MTB/RIF assay was performed as described previously. (WHO 2017, Reje et al., 2015).

Sputum samples are treated with sample reagent (SR) containing NaOH and isopropanol. The SR is added using a 2 to 1 ratio of the sputum sample, homogenized and incubated for 15 min at room temperature. The treated sample is transferred into the cartridge, the cartridge is loaded into the Gene X pert instrument, and an automatic process completes the remaining assay steps.

The system automatically interpreted all results from the measured fluorescent signal into the following categories: invalid, if PCR inhibitors were detected with amplification
failure, negative or positive. Positive results were scaled into 4 categories (very low, low, medium, high) depending on bacterial load and defined susceptible or resistant to rifampicin depending on detection of mutations in RNA polymerase gene.

3. RESULTS AND DISCUSSION

Mycobacterium tuberculosis has been considered as the leading cause of huge threat upon millions population died annually, the critical significant of Gene x pert has been greatly substantiated worldwide owing to the critical key role used in 23 00000 tests in 130 countries via WHO. (Dereje et al., 2015).

The evolving mission of WHO with reach 2035 is to reduce 90% in incidence and 95% in mortality among TB patient. (Wu et al., 2017).

ZN staining was done for 250 samples of the patients who were having a history suggestive of pulmonary tuberculosis. Out of these 75 (30%) sputum samples were AFB smear positive, and 175 (70%) were negative. Then all positive samples were performed on Gene x pert ® MTB/RIF assay. Out of the 250 sputum samples 85 (34%) were MTB (Mycobacterium tuberculosis) positive and 165 (66%) were negative. The results of Gene x pert and ZN staining are compared in our study. It is evident from the (table 1) that Gene x pert MTB/RIF is more useful than ZN staining. As compared to ZN staining it can detect MTB even in 1ml of sputum. The second important advantage of Gene x pert is that it also detects (RIF) rifampicin resistance and helps us to diagnose multidrug resistance tuberculosis (MDR TB). (Meyer et al., 2017).

Table 1: Comparison of sputum between the detection of tuberculosis by smear microscopy and X pert® MTB/RIF

| Gene x pert | AFB +ve | AFB -ve | Total |
|-------------|--------|--------|-------|
| +ve         | 75     | 10     | 85    |
| -ve         | 0      | 165    | 165   |
| Total       | 75     | 175    | 250   |

Sensitivity=100% Specificity=94.29 %

For the years 2017 to 2018 more negative results were found by Gene x pert because during this period even the negative microscopically cases had been tested as a routine without a clinical basis. Although X pert showed high overall sensitivity and specificity with pulmonary samples, its sensitivity has been lower with smear-negative pulmonary samples. (Ngayen et al., 2016), Eutkemeyer et al., 2016).

The Gene X pert MTB/RIF test had a fair sensitivity and specificity for diagnosing smear-negative pulmonary TB. It may be useful for diagnosing pulmonary TB in patients with a negative sputum AFB smear. The assay is faster than culture and can detect rifampicin resistant strains of MTB. (Chakravorty et al., 2017).

Combing smear microscopy and X pert MTB/RIF appears to be an accurate and cost-effective tool for the early diagnosis of pulmonary TB. (Xiaofu et al., 2018).

Gene x pert plays a pivotal role in identifying multi drug resistance tuberculosis and rifampicin resistance. In this study, we find seven drug-resistant patients as illustrated in (table 2) out of 250 (2.8%) cases being confirmed as drug-susceptible TB cases.
Drug-resistant TB poses a huge threat and issue-related fatalities. (Singh et al., 2016, Walters et al., 2017).

The main obstacles for the occurrence and sustained acquisition of multi-drug resistant cases due to a shortage of diagnostic equipment’s, they are expensive, not effective and time consuming. WHO 2018).

The sensitivity of my Xpert MTB/RIF study was 100%, whereby the sensitivity observed in Lima and Pero studies was as high as 96.7% and 100 % in New-Zealand and which was consistent with the findings of this study (Griquiry et al., 2012 ,Williamson et al., 2012)

|                | MTB +ve | MTB -ve | Total |
|----------------|---------|---------|-------|
| RIF not resistant | 78      | 165     | 243   |
| RIF resistant    | 7       | 0       | 7     |
| Total            | 85      | 165     | 250   |

The specificity of X pert MTB/RIF assay found in our study was 94.29%. The result of specificity is comparable with other studies conducted in different places which have shown a specificity ranging from 94.1 to 100 (Balcha et al., 2014 Ausse et al., 2011).

4. CONCLUSIONS

Gene x pert is entirely conclusive and informative comparable to sputum AFB microscopy also the former is sensitive and specific for rifampicin resistance in our lab.

Personnel can obtain minimal training on its utility and secondly the imprudent use of anti-tuberculosis drugs is withheld. Industrialised and non-industrialised communities hailed the early identification of MDR/RIF T.B has dramatically decreased the erroneous results and achieved higher survival among TB patients and eradicates the sources of TB transmission by implementing excellent diagnosis and cure.

Conflict of Interest

There is no conflict of interest.

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