Detecting *Mycobacterium Tuberculosis* through Bacterioferritin Gene Amplification by Real-Time PCR

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ABSTRACT

Tuberculosis is known to be the second most common cause from substantial morbidity and mortality through infectious diseases worldwide following the human HIV Sample selection was performed at the Chest and Respiratory Diseases Institute / Baghdad Medical City in Bagdad. The samples collecting were done from January to October 2019, at which time 90 suspected TB patients were investigated. The results revealed when comparing between RT-PCR test and microbiological test we have calculated sensitivity and specificity for each test. The RT-PCR test showed 100 percent for both sensitivity and specificity while the Microbiological tests showed 0.067 specificity and 0.933 sensitivity. These findings our results showed a high sensitivity and specificity of RT-PCR technique targeting BfrB gene for the detection of MTB by using specific probe.

INTRODUCTION

Infection with Tuberculosis (TB) consider as a contagious disease; the most TB human cases are attributed to Mycobacterium tuberculosis (MTB), which is an acid-fast, non-motile bacterium. Pulmonary TB is the most common type from infection, but some other sites can as well be impacted. Bactria is transmitted through the air from patients with pulmonary TB. It is therefore essential to diagnose MTB with pulmonary samples in order to prevent the spread from disease. [1, 2, 3] Not everybody who infected develops asymptomatic illness, latent infection is quite common, although one in ten latent infections can develop to active TB disease, which if left untreated, kills more than half of its victims. [6]. diagnosis of tuberculosis depends on history, physical examination of patient, chest X-ray, tuberculin skin test, Microscopic smears and culture, molecular technique, and histopathological examination [7]. Tuberculosis is a major health problem worldwide. In 2011 World health organization (WHO) estimated that M. tuberculosis infect arounf 2 billion people (about one third of world populatin), and 2 million deaths occur per year resulted from 9 million new TB cases a year. Ferritins are pervasive in bacteria which can be found in two forms either bacterial ferritin (Ftn) or bacterioferritin (Bfr) (37). The two forms of ferritins possess a dinuclear ferroxidase center, and the difference between the two is that there is no heme-binding site in Ftn but instead this site it has a metal-binding site at the ferroxidase base. (2, 3). Three Bfr formulas have been examined: Bfr, Bfrш, as well as Bfrβ (1, 10, 11, 23, 26). Bfr5-007 differ from Bfr and Bfrβ as it has no metal residue at site 52 formed in heme binding, (5). both Bfr and Bfrβ possess the same set of functional motifs and just because the phylogenetic divergence of their core structure they are seperated (23).

MATERIALS AND METHODS

Samples Collection and DNA Extraction

During January - August 2020 , 90 paitients suspected to have pulmonary tuberculosis lesions have been visted the Institute of-Chest and Respiratory Diseases- Medical City located in Baghdad. Each randomly selected patient asked to inhale deeply about 4 times, cough out and spit thesputum into sterile container. And those collected samples were stored at –20°C until use [9, 10]. Ziehl-Neelsen Stain sputum smears was investigated for the existence of rapid bacilli of pulmonary acid. Of these, 30 specimens were acid quick bacilli with positive pulmonary tuberculosis and 30 specimens were collected as negative control. The DNA extraction is carried out according to the development instructions of Quick-DNATM Miniprep (Zymo – USA).

A set of primer were used to amplify the BfrB gene. The mixture were prepared by adding 0.6 μL of each forward and reverse then 0.5 μL of the probe and 10 μL master mix, 3 μL of eluted DNA then the volume were completed by adding 5.6 μL of distilled water. The thermal cycling program were as follow, enzyme activation 94 C for 6 min, and then followed by 35 cycles of two steps the first one was denaturation 95 C for 20 sec and second step of annealing and florescence screening for 20 sec (55 C) and extension for 20 sec.

RESULTS

Figure 1 showed the primers forward CGGTCGAGGAACGAAACCAT, reverse TCTACGCCGGGAATTTCGAC and probe FAM-CCTGCTCGACCGCGACCTTC-BHQ aligning on the bacterioferritin gene.
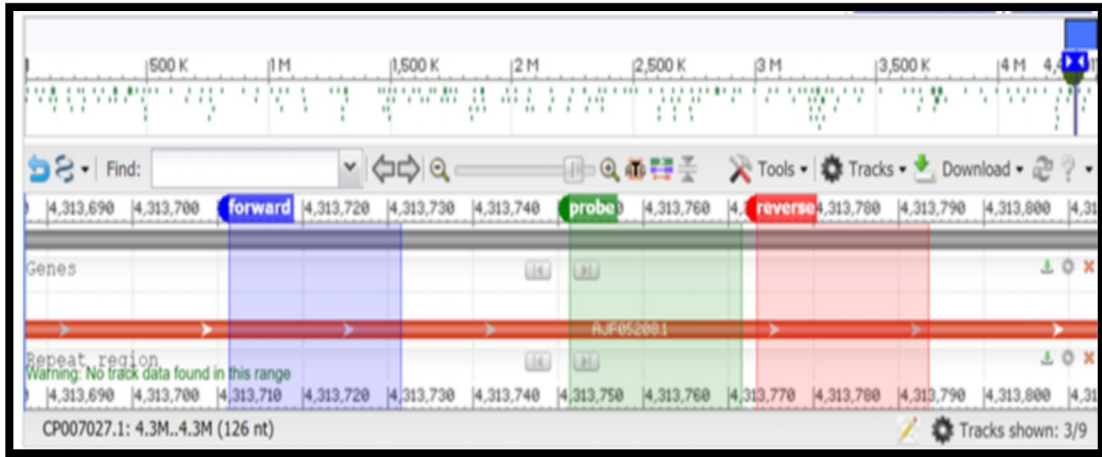
Figure 1: Aligning the primer set and internal oligonucleotides.

The results in figure (2) showing the end results curves of RT-PCR which resulted by the amplification of bacterioferritin gene, and each curve represent the amplification of each sample.
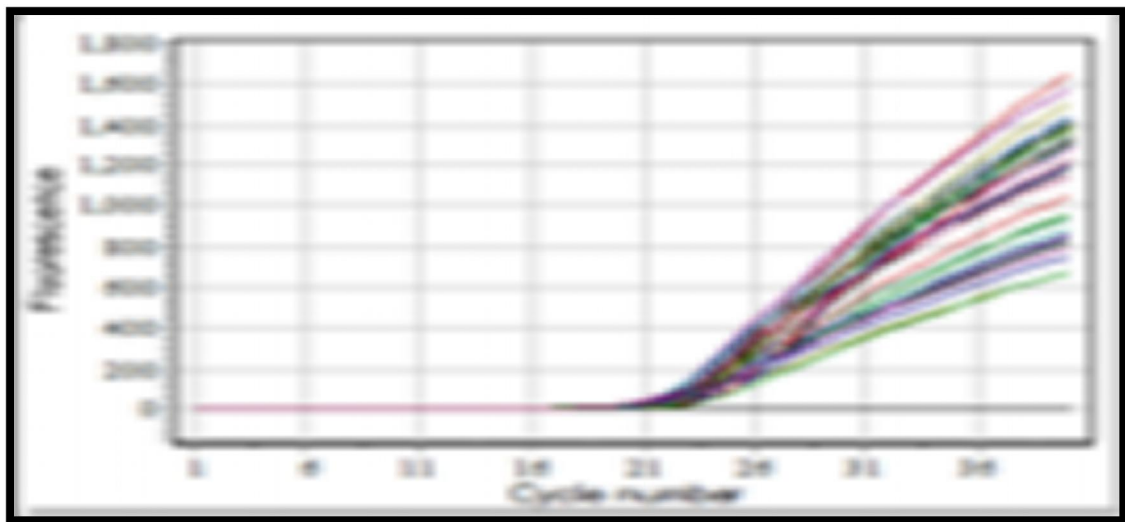

Figure 2: RT-PCR curves for the 30 samples.

The scheme in figure (3) represent the distribution of samples results for both RT-PCR test and microbiological examination tests. 30 true positive samples recorded by RT-PCR and all the healthy samples showed true negative results, while the microbiological examination tests showed 5 false negative results and 2 false positive samples.
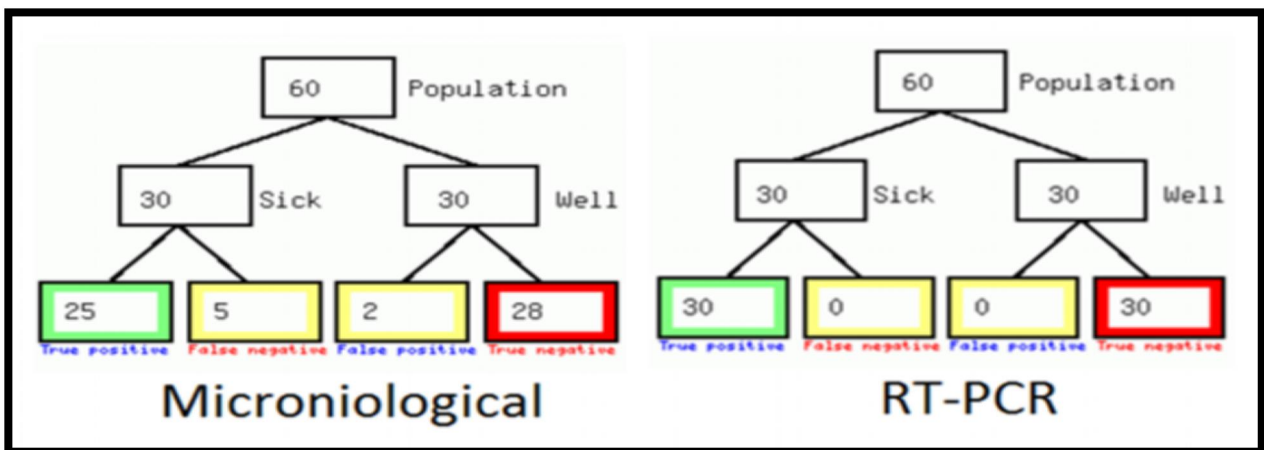

Figure 3: samples distribution between RT-PCR test and microbiological tests.

In order to compere between RT-PCR test and microbiological test we have calculated sensitivity and specificity for each test as shown in table (1). The RT-PCR test showed 100 percent for both sensitivity and specificity while the Microbiological tests showed 0.067 specificity and 0.933 sensitivity.

Table 1: Distribution of samples results between tests type and the characteristics of each test.

Tests		Patients	Control	sensitivity	specificity
RT-PCR	Test positive	30	0	1	1
	Test negative	0	30		
Microbiological tests	Test positive	28	2	0.933	0.067
	Test negative	2	28		

DISCUSSION

Diagnosis of tuberculosis mainly achieved by microbiological culture but nowadays it has become an urgent case to develop a techniques to detect it directly from tissue rapidly and accurately, and this study aim to use bacterioferritin (BfrB) gene for the detection of Mycobacterium tuberculosis. Also, in this study negative samples have been included to test the specificity. A previous study done by Lorente-Leal *et al.*, [15] and they had develop, optimize and validate of a Real-Time PCR by aiming gene named *mpb70* to detect MTBC affiliates in biopsies.

Our results showed a high sensitivity and specificity of RT-PCR technique targeting BfrB gene for the detection of MTB by using specific probe.

In a study conducted by Abbadi, the diagnosis of positive ZN-smears by RT- PCR showed that a higher number of positive ZN-smears belong to the Mycobacterium family. The findings showed that the 48 samples (85.72 per cent) were positive as M. Tuberculosis , and two samples showed two positives results for two bacteria (3.57 percent) M. Tuberculosis, M. Bovis, not M. Bovis BCG was detected while 6 samples showed (10.71%) negative results. Those results indicate that M. Bovis plays a minor role in contrast to M. tuberculosis. The findings in Bagdad have been consistent with the research in Egypt [16], Which was found to be among the 45 M. Tuberculosis complex isolates from sputum samples, 44 of which were classified as M. tuberculosis and one like M.bovis . In comparison to the analysis that showed the absence of M.bovis In Iraq [17]. Difference between the number of positive patient samples when diagnosed with Ziehl Neelsen staining test and RT- PCR technique may be attributable to direct frottis microscopy cannot discriminate between M. Tuberculosis (MTB) and Mycobacterium other than tuberculosis (MOTT) and real-time PCR have a higher specificity and sensitivity than Ziehl Neelsen staining[18].

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