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Full Length Research Paper

Polymerase chain reaction and Quanti FERON enhance acid-fast bacillus stain-based rapid detection of *Mycobacterium tuberculosis* isolated from displaced Iraqi patients

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For exactly detection of the TB cases recently a novel polymerase chain reaction (PCR) based diagnostic kit has been developed. It is based on the nucleic acid amplification (NAA) of specific region of *Mycobacterium* DNA. Quanti FERON-TB test, (QFT) an in vitro diagnostic test that measures a constituents of cell-mediated immune reactivity to *M. tuberculosis* was approved by food and Drug administration (FAD) as an aid for identifying *Mycobacterium tuberculosis* infection. In the current study there were 50 patients (18 displaced and 32 non displaced TB patients) and 40 healthy control. The patient were examined for the presence of TB utilizing Quanti FERON-TB Gold In-Tube assay, polymerase chain reaction (PCR), AFB smear and T.B. culture. It was found that the frequency of positivity of acid-fast stain, culture and Quanti FERON for displaced and non-displaced patients was 36 , 33.3 and 100 and 64 , 66.7 and 100 % respectively. The positivity towards polymerase chain reaction for primers IS6110 and MPB64 for displaced patients was 37.5 and 100% respectively whereas for non displaced patients was 14.3 and 100 % respectively too. The PCR test for the presence of primer MPB64 and Quanti FERON test were 100% positive for all mycobacterial isolates tested from displaced and non displaced patients whereas other identification tests revealed variations in reproducibility.

Keywords: Acid-fast bacilli detection, PCR, Quanti FERON, Culture, Iraqi refugees.

INTRODUCTION

Rapid detection of the *Mycobacterium tuberculosis* complex is important in patient management in terms of initiating appropriate antimicrobial therapy as well as controlling the spread of the pathogen. Although mycobacterial culture continues to be a valuable diagnostic tool, the results are not rapid (Al-Jebouri and Wahid, 2014; Tang et al., 2004). *Mycobacterium* is a genus of weakly Gram-positive bacilli when stained by Gram-stain while demonstrates the staining characteristic of acid-fastness when stained by Ziehl-Neelsen (ZN) method. For exactly detection of the TB cases recently a novel polymerase chain reaction (PCR) based diagnostic kit has been developed. It is based on the nucleic acid amplification (NAA) of specific region of Mycobacterium DNA. These tests are used to rapidly identify the microorganisms in any category of a specimen. The test can reliably detect *M. tuberculosis* bacilli in specimens in hours when compared

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to a week or more time for culture methods (Nuorala, 2004; Alexander et al., 2001). Besides PCR, some another tests involving use of beacons for rapid identification of tuberculosis as well as the mutations associated with drug resistance have been recorded. Quanti FERON-TB test, (QFT) an in vitro diagnostic test that measures a constituents of cell-mediated immune reactivity to *M. tuberculosis* was approved by food and Drug administration (FDA) as an aid for identifying latent *Mycobacterium tuberculosis* infection (Taylar, 2005; Kaul, 2001).The test is based on the quantification of interferon-gamma (IFN-γ) released from sensitized T-lymphocytes in the blood incubated overnight with purified protein derivative (PPD) from *M. tuberculosis* and control antigens (Hershkovitz, 2008; Tokars et al., 1998).

MATERIALS AND METHODS

Patient's Study

This study was carried out in the Consultant Clinic for Respiratory Disease in Kirkuk, since January 2015 to December 2015 included 50 patients (28 males and 22 females) referred to the laboratory of Consultant Clinic for Respiratory Disease in Kirkuk, from different residency were examined, diagnosed with pulmonary tuberculosis based upon TB history, clinical examination and radiological examination. All cases were confirmed by sputum acid fast bacilli (AFB) staining microscopically investigated and obtaining sputum cultures of *Mycobacterium tuberculosis* (Al-Jebouri and Wahid, 2014; Cruickshank et al., 1975), while the control groups were 40 males' healthy individuals of Kirkuk main blood banking donors, they showed no acid fast bacilli and Quanti FERON test negative. The Quanti FERON-TB Gold IT (QFT-GIT) test was performed with commercially available package insert from (Cellestis Limited, Carnegie, Victoria, Australia). The QFT-GIT system used specialized blood collection tubes, which were used to collect whole blood. The QFT-GIT test was performed in two stages. First, whole blood was collected into each of the QFT blood collection tubes, which included a Nil control tube, TB Antigen tube, and a Mitogen Control tube (Al-Jebouri and Wahid, 2014). Polymerase chain reaction (PCR) and mycobacterial DNA was extracted by using Wizard Genomic DNA purification (Promega Co, USA) automated according to manufacturer instructions (Tang et al., 2004).

RESULTS

Relationship between polymerase chain reaction (PCR) result of two genes of *M. tuberculosis* among patients with incidence of tuberculosis of Kirkuk district.

Table 1 reveals the relationship between Polymerase chain reaction (PCR) results of two genes of *Mycobacterium tuberculosis* among patients with tuberculosis in the present study. The results showed that 2(25%) and 3(21.43%) positive using primer gene IS6110, whereas 8(100%) and 14(100%) were positive using primer gene MPB64 among displaced and non-displaced groups respectively. Statistically, there was a non-significant difference between displacement of patients rejoin and the type of incidence tuberculosis gene (p=0.879).

Table 1. Relationship between Polymerase chain reaction result of two genes of Mycobacterium tuberculosis among patients with incidence of tuberculosis of Kirkuk district according to origin of patient.

Type of primer	TB patients n=22				
	Displaced =8		Non-displaced n=14		Total
	No. +ve	%	No. +ve	%	
IS 6110	2	25	3	21.43	5
MPB 64	8	100	14	100	22
Total	10	45.5	17	77.3	27

χ^2= 2.924, p-value = 0.872

Comparison of amplifications results after extraction of DNA from sputum culture growth on LJ medium.

Table 2 represents comparison results of AFB 18(46%), 32(64%), LJ medium culture 10(33.3%), 20(66.7%), Quanti FERON-TB test 18(36%), 32(64%) and amplifications results after extraction of DNA from sputum culture isolates by PCR used primer (IS6110) 3(37.5%), 2(14.3%) and primer (MPB64) 8(100%), 14(100%) in displaced and non-displaced TB patients positivity according to origin of patient. Statistically, results revealed that there were non-significant differences between used methods of TB identification and TB patients origin (p = 0.849).

Table 2. AFB, LJ medium culture, Quanti FERON-TB Gold in tube (QFT-GIT) and PCR (primer IS6110 and MPB64) positivity proportion in sputum and sputum culture on LJ medium specimens according to origin of patient with incidence of tuberculosis.

Origin of TB patient	No. of specimens n=50	AFB Smear n=50		LJ Culture n=50		QFT-GIT n=50		IS6110 primer n=22		MPB64 primer n=22	
		+ve	%	+ve	%	+ve	%	+ve	%	+ve	%
Displaced	18	18	36	10	33.3	18	36	3(8)	37.5	8	100
Non-displaced	32	32	64	20	66.7	32	64	2(14)	14.3	14	100
Total	50	50	100	30	60.0	50	100	5(22)	22.7	22	100

AFB (acid fast bacilli, LJ (Lowenstein Jensen medium), QFT-GIT (Quanti FERON-TB Gold in tube). χ^2= 1.372, p-value = 0.849

DISCUSSION

Polymerase chain reaction methods targeting IS6110 and MPB64 have potential to detect *M. tuberculosis* specific DNA which used in the present study. Various degrees of reactivity in each of PCRs were found by applying two PCR assays to these samples from patients with AFB positive and growth culture on LJ medium. The overall higher proportion of positive results were detected with PCR assay targeting MPB64 by which 8(100%) and 14(100%) showed positive results in both displaced and non-displaced groups respectively as compared to PCR assay targeting IS6110 by which 2(25%) and 3(21.43%) in both displaced and non-displaced groups. The percentage of positivity for amplification of123-bp fragment of target IS6110 in the present study in both displaced and non-displaced groups was lower than that reported elsewhere (Negi et al., 2007). On the other hand, this result of the PCR targeting MPB64 primer was almost similar to that carried out by Asthana *et al.,* (2015) who detected *M. tuberculosis* in clinical samples and found highly specific for the *M. tuberculosis* complex. There was non-significant difference according to displacement of patients and the type of incidence tuberculosis gene (p=0.872). However, PCR has a potentially important role in diagnosis of TB especially targeting MPB64. The positive signals obtained in these cases were higher with MPB64 than with IS 6110 system (Tang, 2004). This could be due to absence of IS6110 copies, which is known to exist in Iraqian *M. tuberculosis* strains. Moreover, this fact was almost similar to what was concluded by Al-Jebouri and Wahid (2014) who found only 10 patients were positive among 19 subjected to PCR using IS 6110 primer gene.

The test positivity percentage of among displaced patients with AFB, culture, Quanti FERON, PCR(IS6110) and PCR(MPB64) was 36,33.3,36,37.5 and 100 respectively, whereas among non displaced patients, the percentage was 64,66.7,64,14.3 and 100 respectively (Table 2).The results presented here revealed that there were significant interactions between used methods of TB identification and the origin of TB patients (p = 0.0275). The use of MPB64 PCR may be better than IS6110 PCR which has a limitation of low or zero copy number in some isolates (Singh et al., 2016). These results suggest need to choose the correct primers for investigations to obtaining the highest results. The primers MPB64 used in this study proved to be specific and should hold promise for the future (Singh et al., 2006; Ritambhara et al., 2014).

CONCLUSIONS

Quanti FERON-TB Gold-In Tube test had high sensitivity with AFB smears. The higher proportion of positive results was detected with PCR assay targeting MPB64 as compared to PCR assay targeting IS6110.Regarding the residence and displacement of the patients origin; the highest positive results were found among rural area in displaced group. Whereas the highest rate were found among urban area in non-displaced population.

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