Molecular study of antibiotic resistance *Mycobacteriumtuberculosis* isolated from clinical samples in Baghdad city

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Abstract

Background: Drug-resistant TB is the most important issue that threatens to interrupt the improvements achieved in tuberculosis control. Multidrug-resistant (MDR) tuberculosis is defined as resistance to both rifampicin and isoniazid.

Objectives: To find molecular feature via DNA extraction for genes in charge of drug resistance in pulmonary tuberculosis patients with hyperglycemia and role of the Xpert MTB/RIF Ultra molecular test efficacy in DR-TB cases detection.

Method: 106 pulmonary TB patient sputum and blood samples were collected in the Institute of Chest and Respiratory diseases in Baghdad and Medical City Hospital from December 2018 - July 2019, 52 were drug resistances TB patients. All patients were examined by conventional method such as direct examination (AFB test), Gene-Xpert test, FBS and HbA1c test were carried out for all cases.

Results: GeneXpert/ultra results revealed 43 cases were Rif resistance in which it was highly significant. The GeneXpert/ultra quantity was mostly medium in the bacillary load. The Rif's resistance cases were mostly registered in the age group (20-59) and in male population. The most frequent patient's type was New MDR. The GeneXpert/ultra quantity and HbA1c levels results showed that most elevated HbA1c cases had a medium to high bacillary load. The DST results were significant in which 95.3% were Rif's resistance and 62.8% were STR's resistance. The LPA on 23 DR cases showed the following; 13 patients were MDR cases in which a deletion in the band WT8 and mutation in the MUT3 band of the gene rpoB and deletion in the inhA WT1 band of the inhA gene were the most notice.

Conclusions: Molecular method had showed that mutations in the *rpoB* gene especially 530 and S531L codon are responsible for majority of RMP resistance in MTB while mutations at the -15/-16 *InhA* codon and 315 of *KatG* codon responsible for most INH-resistance cases.

Keywords: *Antibiotic resistance; Mycobacteriumtuberculosis: clinical samples.*

Introduction

Tuberculosis (TB) is the leading cause of death due to an identifiable infectious pathogen worldwide[(1)]. TB is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS). Millions of people continue to fall sick with TB each year[(2)]. Drug resistance is a natural phenomenon increased by man. Resistance is initiated by a genetic mutation that makes a drug unsuccessful against the mutant bacteria. There is an increased risk of developing resistant bacteria in patients with a large bacillary load because more spontaneous mutations arise in a big population. Then the inadequate treatment permits for

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the selection of a drug-resistant strain to become the dominant strain in patients infected with TB, which can spread from person to person in the same manner as drug-sensitive TB[3]. Multidrug-resistant (MDR) TB is defined as disease due to M. tuberculosis resistant to at least rifampin (R) and isoniazid (H), the vital elements of TB treatment(4). MDR-TB progresses in otherwise curable TB when the course of antibiotics is interrupted and the levels of the drug in the body are inadequate to kill the bacteria 100%[6]. An estimated 600,000 individuals infected with multidrug-resistant (MDR) forms of tuberculosis (TB) in 2016, including 110,000 with rifampicin-resistant TB (RR-TB). Only 153,000 cases of MDR-TB were identified and only 130,000 people began MDR-TB treatment. An expected 240,000 individuals deceased from MDR/RR-TB in 2016[7]. Multi drug resistant TB is a serious public health problem, as it has been associated with higher rates of failure and mortality than drug-susceptible TB, especially in human immunodeficiency virus (HIV) confected patients[8].

Method

Study was conducted at National Reference Laboratory of Tuberculosis/Baghdad from December 2018 to July 2019. 106 TB specimens were collected in this study, 54 of them were new cases and sensitive for RIF and INH; while the other 52 of specimens were DR, 30 of those 52 were hyperglycemia. Their history was taken including the sex, age, history of the symptoms.

The direct examination (AFB) was done by Ziehl-Neelsen stain. The stain was prepared according to the guidelines of laboratory manual guidelines in Iraq[11], the culturing was done on Lowenstein-jensen medium (L.J) solid media, while drug susceptibility test was also carried as instructed in the Laboratory manual guidelines in Iraq(11). Molecular Method for Screening Drug-Resistant TB [12] included **Gene Xpert System MTB/ RMP or Xpert MTB/RIF Ultra**(13-16). and **Line Probe Assays**[17] the Genotype MTBDR *plus* test (Hain Lifescience GmbH, Nehren, Germany)[18] is based on the DNA-strep technology[19] in which they were carried outaccording to the guidelines of laboratory manual guidelines in Iraq.[11]

Result

106 TB specimens were collected in this study, 54 of them were new cases and sensitive for RIF and INH; 19 female and 35 male, while the other 52 of specimens were DR cases 14 female and 38 male, 30 of those 52 were hyperglycemia. The patients were arranged according to age into four groups.

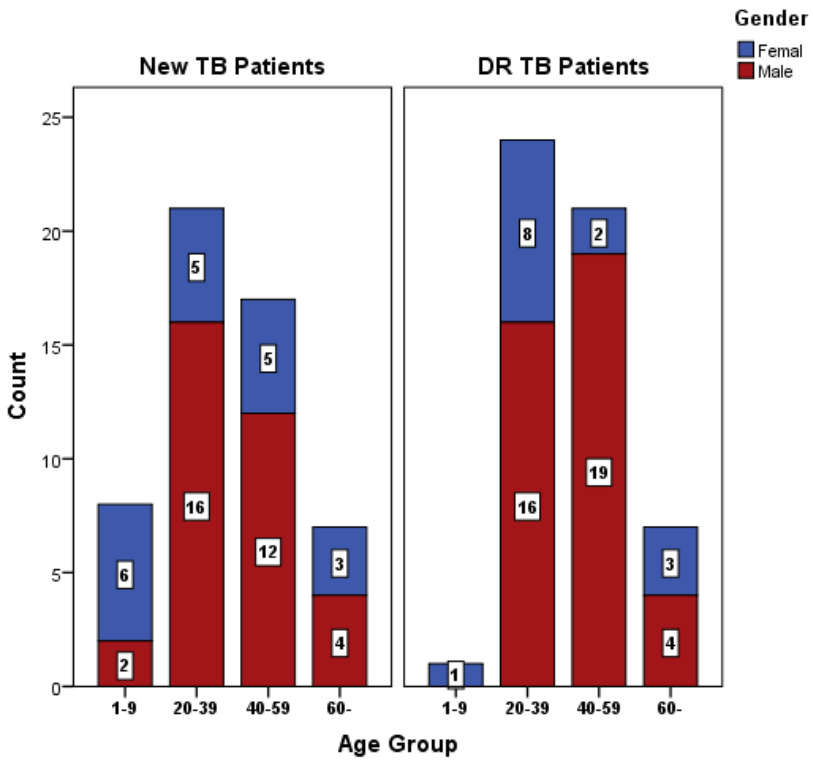

Figure (1): The age group and gender distrubution acording to the TB type

In this study the most recorded cases were in the age groups of (20-38)y in which (38.9%) patients were new TB cases while (46.2%) were DR TB cases and the age group of (39-57)y in which (33.3%) patients were new TB cases while (38.5%) patients were DR TB cases. The P value of age was (0.126) and the mean age of patients was (40.4) years old. Males subjects were more innumerable than female in TB cases according to results, which showed that males were 35 (64.8%) in New TB cases (control), 19 (35.2%) in DR TB cases.

Table (1): Distribution of DR-TB patients according to their DST results:

		New DR		Retreat DR		New MDR		Retreat MDR	
		N=5	100%	N=10	100%	N=21	100%	N=16	100%
Streptomycin	Resistant	2	40.0%	3	30.0%	12	57.1%	12	75.0%
	Sensitive	3	60.0%	7	70.0%	9	42.9%	4	25.0%
Isoniazid	Resistant	1	20.0%	9	90.0%	21	100.0%	16	100.0%
	Sensitive	4	80.0%	1	10.0%	0	0.0%	0	0.0%
Rifampicin	Resistant	4	80.0%	1	10.0%	19	90.5%	16	100.0%
	Sensitive	1	20.0%	9	90.0%	2	9.5%	0	0.0%
Ethambutol	Resistant	2	40.0%	3	30.0%	11	52.4%	12	75.0%
	Sensitive	3	60.0%	7	70.0%	10	47.6%	4	25.0%

Out of the 52 cases ((21/52) (40.38%) new MDR, (16/52) (30.76%) retreated MDR.

The 5 new DR cases (4/5) (80%) RIF's resistance. While the retreated DR cases showed that (9/10) (90%) were INH resistance. The new MDR cases were 21 in which (21/21) (100%) were INH resistance, (19/21) (90.5%) were RIF's resistance. The 16 retreated MDR cases (16) (100%) were INH resistance, (16) (100%) were RIF resistance.

Table (2): The Gxpert/Ultra results distribution according to age, gender, type, HbA1c Level, Zeheel Nelson and Gxpert/Ultra Quantity:

	GeneXpert/Ultra Result				P value
	RR		RS		
Variables	N=43	100%	N=63	100%	
Age Group					0.256
< 20 y	1	2.3%	8	12.7%	
20-39	20	46.5%	25	39.7%	
40-59	15	34.9%	23	36.5%	
≥ 60y	7	16.3%	7	11.1%	
Gender					0.869
Female	13	30.2%	20	31.7%	
Male	30	69.8%	43	68.3%	
Type					<0.001
New	0	0.0%	53	84.1%	
Retreat	0	0.0%	1	1.6%	
New DR	4	9.3%	1	1.6%	

	GeneXpert/Ultra Result				P value
	RR		RS		
Variables	**N=43**	**100%**	**N=63**	**100%**	
Retreat DR	2	4.7%	8	12.7%	
New MDR	21	48.8%	0	0.0%	
Retreat MDR	16	37.2%	0	0.0%	
HbA1c Level					<0.001
Normal(4.6-6.5)	18	41.9%	58	92.1%	
Abnormal	25	58.1%	5	7.9%	
Zeheel Nelson					0.147
Positive	43	100.0%	60	95.2%	
Negative	0	0.0%	3	4.8%	
GeneXpert/Ultra Quantity					0.016
High	17	39.5%	10	15.9%	
Medium	17	39.5%	35	55.6%	
Low	8	18.6%	10	15.9%	
Very low/Scanty	1	2.3%	8	12.7%	

The study is showing that the most age group cases with RR lies in the (20-39) group as 20(46.5%) patients followed by the (40-59) group of TB patients by 15(34.9%) The male patients are also dominant in number in the both RR cases as well as the RS cases, males 30 (69.8%) out of 43 cases with RR results. The 43 RR cases present most patients are new MDR with 21 (48.8%). While the 63 RS cases mostly are new TB patients with 53(84.1%), 8 (12.7%) retarded DR cases and 1(1.6%) retreated and new DR respectively.

Table (3): The Gxpert/Ultra results distribution according to the TB patients symptoms:

	GeneXpert/Ultra Result				P value
	RR		RS		
Variables	**N=43**	**100%**	**N=63**	**100%**	
Productive cough					0.011
Present	26	60.5%	52	82.5%	
Absent	17	39.5%	11	17.5%	
Night Sweating Fever					<0.001
Present	4	9.3%	41	65.1%	
Absent	39	90.7%	22	34.9%	
Weakness					0.134
Present	31	72.1%	53	84.1%	
Absent	12	27.9%	10	15.9%	
weight loss					0.609
Present	28	65.1%	44	69.8%	
Absent	15	34.9%	19	30.2%	
Hemoptysis					0.343
Present	3	7.0%	8	12.7%	
Absent	40	93.0%	55	87.3%	

The night sweating fever were the most symptom with significant P < 0.001 followed closely by productive cough symptom the P value being 0.011. While the most common symptoms were weakness present in 31 (72.1%) RR cases and 53 (84.1%) RS cases, P value 0.134.

Table (4): Gexp/Ultra distribution according to culture on LG and DST (SIRE) results

Variables	GeneXpert/Ultra Result				P value
	RR		RS		
	N=43	100%	N=63	100%	
Culture on LG					---
Positive	43	100.0%	10	100.0%	
Negative	0	0.0%	0	0.0%	
Streptomycin					0.014
Resistance	27	62.8%	2	20.0%	
Sensitive	16	37.2%	8	80.0%	
Isoniazid					0.884
Resistance	38	88.4%	9	90.0%	
Sensitive	5	11.6%	1	10.0%	
Rifampicin					<0.001
Resistance	41	95.3%	0	0.0%	
Sensitive	2	4.7%	10	100.0%	
Ethambutol					0.108
Resistance	25	58.1%	3	30.0%	
Sensitive	18	41.9%	7	70.0%	

All the 53 samples culture result were positive except with 3 of them were subjected to contamination and had to be re-cultured. DST culture results for RIF resist showed 41 (95.3%) positive results on LG out of the 43 RR Gexp/Ultra cases with highly significant P value < 0.001.

Table (5): The LPA results distribution according to the TB patients types:

LPA	Retreat		New DR		Retreat DR		New MDR		Retreat MDR	
	N=0	100%	N=3	100%	N=7	100%	N=7	100%	N=6	100%
rpoB	1	100.0%	3	100.0%	7	100.0%	7	100.0%	6	100.0%
rpoBWT1	1	100.0%	3	100.0%	7	100.0%	7	100.0%	6	100.0%
rpoBWT2	1	100.0%	2	66.7%	7	100.0%	7	100.0%	6	100.0%
rpoBWT3	1	100.0%	2	66.7%	7	100.0%	7	100.0%	6	100.0%
rpoBWT4	1	100.0%	2	66.7%	7	100.0%	7	100.0%	6	100.0%
rpoBWT5	1	100.0%	3	100.0%	7	100.0%	7	100.0%	6	100.0%
rpoBWT6	1	100.0%	3	100.0%	7	100.0%	7	100.0%	6	100.0%
rpoBWT7	1	100.0%	3	100.0%	7	100.0%	7	100.0%	5	83.3%
rpoBWT8	1	100.0%	2	66.7%	5	71.4%	1	14.3%	2	33.3%

LPA	Retreat		New DR		Retreat DR		New MDR		Retreat MDR	
	N=0	100%	N=3	100%	N=7	100%	N=7	100%	N=6	100%
rpoBMUT1	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
rpoBMUT2A	0	0.0%	1	33.3%	0	0.0%	0	0.0%	0	0.0%
rpoBMUT2B	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	16.7%
rpoBMUT3	0	0.0%	2	66.7%	2	28.6%	4	57.1%	4	66.7%
katG	1	100.0%	3	100.0%	7	100.0%	7	100.0%	5	83.3%
katGWT	1	100.0%	3	100.0%	4	57.1%	6	85.7%	3	50.0%
katGMUT1	0	0.0%	0	0.0%	3	42.9%	1	14.3%	2	33.3%
katGMUT2	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	16.7%
inhA	1	100.0%	3	100.0%	6	85.7%	7	100.0%	6	100.0%
inhAWT1	1	100.0%	2	66.7%	3	42.9%	2	28.6%	3	50.0%
inhAWT2	1	100.0%	2	66.7%	5	71.4%	5	71.4%	5	83.3%
inhAMUT1	0	0.0%	0	0.0%	1	14.3%	4	57.1%	2	33.3%
inhAMUT2	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
inhAMUT3A	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
inhAMUT3B	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Discussion

The current study showed a broad range of patients' age with tuberculosis which suggests that all ages can be susceptible to the infection with MTB. These results were compatible with that being reported by studies in Baghdad by AL-Khafaji[23] and in Sulaimaniyah, Iraqi Kurdistan (24) and another which involve almost seven countries[13].

The results also showed that the most prevalent age groups are (20-38) and (39-57) years old of TB patients. and this was also found in the other studies in Iraq such as Kareem *et al.,*[25] and study from 2009- 2012 by Al-khazraji.; et al[26], also The results of study expressed by AL-Khafaji in [23] displayed a large-scale age range of TB patients (> 10 to more than 60 years old) and reveal that the predominant age groups were (21-30) and (41-50) years old of TB patients, the low percentage of TB cases among age group less than 14 years may be due to the BCG vaccine which is given at young age, vaccination give good protection for children however this immunity decline with age.

The Difference results showed in this study of TB cases between men and women may reveal physiological differences (i.e. sex differences) in the epidemiology of TB, differences in the social positions of female and male that affects the risk of exposure and/or gender differences in access to care.

The results of the Genexpert/ultra assay with the HbA1c were of highly significant and it goes with results of the study by Ali RH.; et al which conducted in the developed countries, that the most TB cases were found in Europe; it was more prevalent in elder people due to diabetes mellitus and among immune-compromised patients.[34].

Conclusion

Molecular method had showed that mutations in the *rpoB* gene especially 530 and S531L codon are responsible for majority of RMP resistance in MTB while mutations at the -15/-16 *InhA* codon and 315 of *KatG* codon responsible for most INH-resistance cases. Also Diabetes Mellitus is a recognized risk factor for TB (DR-TB) cases in Iraq. TB patients with diabetes mellitus had shown a rather high bacillary load in molecular test. Some DM-TB patients (with uncontrolled DM) may not respond to the anti-TB treatment and more likely to develop into drug resisting (DR) cases.

Conflict of Interest: None

Source of Findings: Self-findings.

Ethical Clearance: None

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