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Brief Communication

Mutation in *katG315* is, possibly, a good prognostic marker for treatment with second-line drugs in multi-drug resistant tuberculosis: A preliminary study

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Abstract

The aim of this study was to explore baseline data, laboratory and molecular analyses to determine if any could serve as potential prognostic marker(s) for treatment response to second line tuberculosis regimens. Of a total number of 50 multi-drug resistant tuberculosis (MDR-TB) patients starting second-line drug MDR-TB treatment in Iraq, only 21 showed treatment adherence and thus, included in this study. Response to treatment was monitored for 11 months by sputum microscopy and culture. We explored baseline data, laboratory and molecular analyses to determine if any could serve as potential prognostic marker(s) for treatment response. Highly significant association (P = 0.019) was detected between mutations in *katG315* codon and good response to second-line anti-TB drugs. Spoligotyping and mycobacterial interspersed repetitive unit variable number tandem repeat confirmed that *katG315*-mutatnt isolates were genotypically unrelated. The *katG315* mutation is a potential prognostic marker for treatment response to second-line anti-tuberculosis drugs. One possible explanation of our results is that the *katG315*-mutants are sensitive to bacterial killing by "oxidative killing."

Key words: *KatG315, multi-drug resistant, second-line drugs, tuberculosis*

Introduction

The most recent World Health Organization report showed that drug resistance tuberculosis (TB) is spreading and may be on the rise.[1] While, previous estimates demonstrated the prevalence of drug resistance at around 5%, a very recent study documented a prevalence up to 10 times higher in some places, where almost half of the patients with infectious disease are transmitting multi-drug resistant (MDR) strains of *Mycobacterium tuberculosis*.[2] MDR and extensive drug resistance (XDR) TB represent the major threat for TB control.[3]

MDR and XDR-TB treatment involve using second-line anti-TB drugs, which are less effective, more toxic and regimens takes longer periods (up to 24 months or more). Delayed detection of treatment failure during the course of those regimens may entails risks of transmitting extremely drug resistance strains and may contribute to the development of strains that would resist almost all known anti-TB drugs. Pre-treatment prognostic markers are needed as they could allow stratification of patients into groups with different treatment requirement and controlling measures. The only validated prognostic marker for the outcome of treatment with a second-line anti-TB drug regimen is the phenotypic drug susceptibility testing (DST) results prior to treatment.[4] Dalton, *et al*. (2012) assessed resistance to second-line anti-TB drugs in eight countries and found that previous treatment with second-line drugs was consistently the strongest risk factor for subsequently resistance to these drugs, which increased the risk of XDR-TB by more than four times.[2] In Iraq, as this is the first time a second-line regimens are being used, it is not possible to assess the previous treatment with second-line drugs as a prognostic marker.

In this study, we aimed to explore the baseline data of patients in addition to genotyping and molecular analyses for clues about possible risk factors for resistance or prognostic markers for response to treatment with a second-line anti-TB regimen.

Subjects and Methods

On June, 2011, a total of 50 MDR-TB patients started treatment with second-line anti-TB drugs at the National Centre of Tuberculosis and Chest illnesses-National Tuberculosis Programme in Baghdad, Iraq. However, 29 patients showed irregular adherence to treatment and follow-up. Only 21 patients showed treatment adherence

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and thus, included in this study. According to the guidelines for the programmatic management of drug resistant, the treatment included an intensive phase of 8 months followed by continuation phase planned to continue for up to 16 months with monthly monitoring by smear microscopy and culture.[4]

Basic demographic data were collected and DST to four first-line anti-TB drugs was performed.[5] Spoligotyping and mycobacterial interspersed repetitive unit variable number tandem repeat (MIRU-VNTR) were used to genotype the isolates.[6,7] Two independent allele-specific polymerase chain reaction (PCR) systems were used to detect the mutations in *katG*315 and *inhAP*-15 codons,[8] whereas, a single-step multiplex allele-specific PCR assay was used to detect mutations in the *rpoB* gene (codons 516,526, and 531).[9]

Results

Detailed data and results are shown in Table 1. A comparison of spoligotyping results with the international spoligotyping database (SpolDB4) showed that 8 isolates belong to T1 sub lineage/group, 7 belong to CAS1-Delhi, 2 belong to H3, 2 belong to H4 (Ural-2), one belongs to the Turkey sub lineage. One did not matching any sub lineages in the international database (SpolDB4) and thus, is designated as unknown sub lineage. Genotyping by the 15 locus MIRU-VNTR method identified a single cluster containing 3 isolates belonging to Shared International Type-1144 (SIT-1144), whereas the rest of the isolates were unique.

Regarding to treatment outcome, 'good response' to treatment with second-line drugs (determined by sputum and culture conversion to negative) was seen in 13 cases (61.9%). A total of 4 cases (19%) showed 'poor response' (determined by consistent smear microscopy and culture positivity along the study period. In addition, 4 (19%) cases showed conversion of smear microscopy and culture to negativity but later on, reverted to positivity.

We compared the available demographic data and results of DST, however, no association could be detected [results are summarized in Table 2]. In addition, no specific association could be found between the *M. tuberculosis* genotypes with the responses to treatment with second-line drugs.

Next, we compared the responses to treatment with the results of allele-specific PCR assays for mutations in *rpoB*, *katG315*, and *inhAP-15*. Surprisingly, we found a significant association between mutations in the *katG315* codon and a 'good response' to treatment with second-line drugs (P = 0.019), where all isolates (n = 8) harbouring *katG315* mutations were shown to respond well to treatment as shown in Table 3. It's worthy to mention that a 'good response' was seen in 13 cases and *katG315* mutations were found in 8/13 (61.54%). However, no significant association was found with mutations in *inhAP-15* or *rpoB*. Moreover, combined spoligotyping and MIRU-VNTR results have revealed that the isolates harbouring mutations in *katG315* codons are unrelated because they are belong to different *M. tuberculosis* SITs.

Discussion

This preliminary reported, for the 1st time, a significant association between *katG315* mutations and 'good response' to treatment with second-line anti-TB drug. If these results are confirmed, then they could have significant implications for and applications in TB treatment and control. One potential argument of our results would be is that the isolates harbouring mutations in *katG315* codons are represent a clone and consequently, the results generated thereof would represent characteristics of this clone rather than a general phenomenon. However, the combined spoligotyping and MIRU-VNTR results revealed that the isolates are unrelated and thus, our results seem not to be confined to a certain clone or sublineages. Indeed, the *katG*-mutant isolates in the current study belong to distinct *M. tuberculosis* sublineages that are globally distributed such CAS1-Delhi, H3 and T1.[6]

The *M. tuberculosis katG* gene encodes a dual function enzyme catalase-peroxidase, which confers sensitivity in *M. tuberculosis* to isoniazid (INH). Mutations in *katG315* are associated with resistance to INH in 50-70% of strains.[10] INH-resistant clinical isolates of *M. tuberculosis* often lose the catalase and peroxidase enzyme encoded by *katG*, especially in high level resistance strains (minimal inhibitory concentration [MIC] >5 μg/ml) (low-level resistance strains [MIC <1 μg/ml] often still possess catalase activity).[11]

One potential mechanistic explanation for our results is could be suggested in the context of 'oxidative killing.' *M. tuberculosis* is a facultative intracellular bacterium and once phagocytosed, the organism resides in a vacuole. Within this vacuole, the organism is exposed several bactericidal mechanisms, including the production of reactive oxygen intermediates. However, *M. tuberculosis* has been shown to have a high resistance to killing by up to millimolar concentrations of H_2O_2. This resistance is believed to be mediated largely by the mycobacterial catalase-peroxidase protein encoded by the gene *katG* and to lesser extent by another gene product of *ahpC*.[12]

Inadequate use of INH in the treatment of TB infections may lead to selection of isoniazid-resistant *katG*-mutant strains (that have lost the *katG* activity). These strains, though resistant to isoniazid, are susceptible to oxidative stress. Manca *et al*. (1999) have shown that strains with no detectable *katG* expression or catalase activity are relatively sensitive to killing by exogenous H_2O_2.[12]

Table 1: Detailed results obtained including demographic, epidemiologic, drug-resistance, and genotyping information on a total of 21 *M. tuberculosis* strains isolated from Iraqi patients before initiation of treatment with second-line drugs

Strain	Sex/age	Isolation city	Spoligotype description	DST	SIT	Lineage*	MIRU-15	15-loci MIT	Treatment regimen	Response	Mutations
2818	F/28	Baghdad-Sadr City 17	■■■□□□□■■■■■■■■■■■■□□□□□□□□□□□□□□□□■■■■■	HRS	22	CAS1-Delhi	274653422455524	Orphan	8 Cm-Cs-Eto-Lfx-Z+E-B6/16 Cs-Eto-Lfx-Z+E-B6	Good	*rpoB516*
4870	F/24	Salah Aldin Province	■■■□□□□■■■■■■■■■■■■□□□□□□□□□□□□□□□□■■■■■	HRE	22	CAS1-Delhi	27475342246-424	Orphan	8 Cm-Cs-Eto-Lfx-Z+E-B6/16 Cs-Eto-Lfx-Z+E-B6	Good	*rpoB531, katG315*
3425	M/30	Musol Province	■■■□□□□■■■■■■■■■■■■□□□□□□□□□□□□□□□□■■■■■	HR	25	CAS1-Delhi	26355422-665324	Orphan	8 Cm-Cs-Eto-Lfx-Z-E-B6/16 Cs-Eto-Lfx-Z-E-B6	Good	*katG315*
5068	F/30	Baghdad-North	■■■□□□□■■■■■■■■■■■■□□□□□□□□□□□□□□□□■■■■■	HRES	25	CAS1-Delhi	234553122485324	Orphan	8 Cm-Cs-Eto-Lfx-Z-B6/16 Cs-Eto-Lfx-Z-B6	Good	*katG315t*
5699	F/19	Misan Province	■■■■■■■■■■■■■■■■■■■■□□□□□■□□■■■■■□□□□■■■■■■■	HRES	41	Turkey	151132342363445	Orphan	8 Am-Cs-Eto-Lfx-Z-B6/16 Cs-Eto-Lfx-Z-B6	Good	*rpoB526, katG315, inhAp-15*
5282	M/35	Baghdad-Suburb-South-East	■■■■■■■■■■■■■■■■■■■■□□□□□□□□□□□□□□□□	HSE	46	Unknown	222638343222225	Orphan	8 Cm-Cs-Eto-Lfx-Z-B6/16 Cs-Eto-Lfx-Z-B6	Reverted/ re-infected	*rpoB531*
4305	M/35	Najaf Province	■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■■■■■■	HRES	50	H3	273534325263243	Orphan	8 Cm-Cs-Eto-Lfx-Z+E-B6/16 Cs-Eto-Lfx-Z+E-B6	Poor	*rpoB531, inhAp-15*
4846	M/49	Kerbala-Province	■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■■■■■■	HR	50	H3	243534337363244	Orphan	8 Cm-Cs-Eto-Lfx-Z-E-B6/16 Cs-Eto-Lfx-Z-E-B6	Good	*rpoB531, katG315, inhAp-15*
2389	F/17	Baghdad-Suburb-South	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■■■■■■■■■□□□□■■■■■■■	HRES	86	T1	22363-3-5292323	Orphan	8 Cm-Cs-Eto-Lfx-Z-B6/16 Cs-Eto-Lfx-Z-B6	Good	*rpoB531, katG315*
4752	M/26	Wasit-Province	■□■■■■■■■■■■■■■■■■■■■■■■■■■■□□□■□□□□■■■■■■■	HRE	127	H4 (Ural-2)	22463134256-223	Orphan	8 Am-Cs-Eto-Lfx-Z-B6/16 Cs-Eto-Lfx-Z-B6	Good	*rpoB531*
5981	F/45	Baghdad-Suburb-East	■□■■■■■■■■■■■■■■■■■■■■■■■■■■□□□■□□□□■■■■■■■	HRS	127	H4 (Ural-2)	224533342562223	Orphan	8 Am-Cs-Eto-Lfx-Z-B6/18 Cs-Eto-Lfx-Z-B6	Good	*rpoB531, katG315t*
2806	M/59	Diwaniya Province	■■■□□□□■■■■■■■■■■■■□□□□□□□□□□□□□□□□■■■■■	HRES	247	CAS1-Delhi	244754322492624	Orphan	8 Am-Cs-Eto-Lfx-Z-B6/16 Cs-Eto-Lfx-Z-B6	Good	*rpoB516, katG315*

Contd...

Table 1: Contd

Strain	Sex/age	Isolation city	Spoligotype description	DST	SIT	Lineage*	MIRU-15	15-loci MIT	Treatment regimen	Response	Mutations
2260	M/30	Diwaniya Province	□□□□■■■■■■■□□■■ ■■■■■■■■■■■■■■■ ■■□□□□■■■■■■■	HRES	284	T1	244632432232224	Orphan	8 Am-Cs-Eto-Lfx-Z-B6/16 Cs-Eto-Lfx-Z-B6	Good	*rpoB516, inhAp-15*
3313	M/35	Sulaimaniya Province	□□□□■■■■■■■□□■■ ■■■■■■■■■■■■■■■ ■■□□□□■■■■■■■	HRES	284	T1	243542432333125	228	8 Am-Cs-Eto-Lfx-Z+E-B6/16 Cs-Eto-Lfx -Z+E-B6	Reverted/ re-infected	*rpoB531*
5159	F/35	Babil Province	■■■□□□□■■■■□■■■ ■■■■■■■□□□□□□□□ □□□□■■■■■■■■■	HRE	954	CAS1-Delhi	264753422485424	Orphan	8 Cm-Cs-Eto-Lfx-Z-B6/16 Cs-Eto-Lfx-Z-B6	Poor	*rpoB531*
3233	M/27	Nasiriya Province	■■■■■■■■■■■■■■■ ■■■■■□□□□□□□■■■ ■■□□□□■■■■■■■	HR	1144	T1	243734244333424	223	8 Am-Cs-Eto-Lfx-Z+E-B6/16 Cs-Eto-Lfx-Z+E-B6	Good	*rpoB531, inhAp-15*
3980	M/25	Najaf Province	■■■■■■■■■■■■■■■ ■■■■■□□□□□□□■■■ ■■□□□□■■■■■■■	HRE	1144	T1	242734244333424	230	8 Cm-Cs-Eto-Lfx-Z-B6/16 Cs-Eto-Lfx-Z-B6	Reverted/ re-infected	*rpoB531, inhAp-15*
4863	F/12	Baghdad-Suburb-East	■■■■■■■■■■■■■■■ ■■■■■□□□□□□□■■■ ■■□□□□■■■■■■■	HRE	1144	T1	242734244333424	230	8 Cm-Cs-Eto-Lfx-Z+E-B6/16 Cs-Eto-Lfx-Z+E-B6	Poor	*rpoB531, inhAp-15*
5700	F/45	Misan Province	■■■■■■■■■■■■■■■ ■■■■■□□□□□□□■■■ ■■□□□□■■■■■■■	HRES	1144	T1	242734244333424	230	8 Am-Cs-Eto-Lfx-Z-B6/16 Cs- Eto-Lfx-Z-B6	Poor	*rpoB531, inhAp-15*
5844	M/20	Baghdad-Central	■■■■■■■■■■■■■■■ ■■■■■□□□□□□□■■■ ■■□□□□■■■■■■■	HRE	1144	T1	252734244333424	222	8 Am-Cs-Eto-Lfx-Z-B6/16 Cs-Eto-Lfx-Z-B6	Reverted/ re-infected	*rpoB531*
5790	M/42	Baghdad-North-East	■■■□□□□■■■■■■■■ ■■■■■□■□□□□□□□□ □□□□□■■■■■■■■■	HRE	2145	CAS1-Delhi	266833432465544	Orphan	8 Am-Cs-Eto-Lfx-Z-B6/16 Cs-Eto-Lfx-Z-B6	Good	*rpoB531t*

*Lineage designation is based on signatures provided in SpolDB4 (Brudey *et al*., 2006). DST: Drug susceptibility testing, SIT: Shared-international-types, *M. tuberculosis*: *Mycobacterium tuberculosis*, MIT: MIRU-international-type, MIRU: Mycobacterial interspersed repetitive units, HR: Resistant to isoniazid and rifampin, HRE: Resistant to isoniazid, rifampin and ethambutol, HRES: Resistant to isoniazid, rifampin, ethambutol and streptomycin, HRS: Resistant to isoniazid, rifampin and streptomycin, HSE: Resistant to isoniazid, streptomycin and ethambutol

Table 2: Correlation of age, gender and DST profile with response to treatment with second-line anti-tuberculosis drugs

Variable	Results of monitoring			Total
	Good response (sputum and culture conversion)	Consistent positivity	Reversion at any time during treatment course	
Age (years)				
Mean (SD)	35 (14.11) years	28 (10.42) years	38.8 (5.25) years	
P value	0.769			
Gender				
Males, no. (%)	7 (50)	3 (21.4)	4 (28.6)	14 (100)
Females, no. (%)	6 (85.7)	1 (14.3)	0	7 (100)
Total (% within gender)	13 (61.9)	4 (19)	4 (19)	21 (100)
P value	0.214			
HR				
No. (% within DST)	3 (100)	0 (0.0)	0 (0.0)	3 (100)
HRE				
No. (% within DST)	3 (42.8)	2 (28.6)	2 (28.6)	7 (100)
HRES				
No. (% within DST)	5 (62.5)	2 (25.0)	1 (12.5)	8 (100)
HRS				
No. (% within DST)	2 (100)	0 (0.0)	0 (0.0)	2 (100)
HSE				
No. (% within DST)	0 (0.0)	0 (0.0)	1 (100)	1 (100)
Total (% within DST)	13 (65)	4 (20)	4 (15)	21 (100)
P value		Low number=could not be assesed		

DST: Drug susceptibility testing, SD: Standard deviation,*HR: Resistant to isoniazid and rifampin, HRE: Resistant to isoniazid, rifampin and ethambutol, HRES: Resistant to isoniazid, rifampin, ethambutol and streptomycin, HRS: Resistant to isoniazid, rifampin and streptomycin, HSE: Resistant to isoniazid, streptomycin and ethambutol

Table 3: Correlations of mutations in *katG315*, *inhAP-15* and *rpoB* with response to treatment with second-line anti-tuberculosis drugs

Variable	Monitoring results			Total
	Good response	Consistent positivity	Reversion at any time during treatment course	
KatG315				
Wild-type, no. (% within *katG315*)	5 (38.5)	4 (30.8)	4 (30.8)	13 (100.0)
Mutated, no. (% within *katG315*)	8 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)
P value (Chi-square)	0.019*			
inhAP-15				
Wild-type, no. (% within *inhAP-15*)	9 (69.2)	1 (7.7)	3 (23.1)	13 (100.0)
Mutated, no. (% within *inhAP-15*)	4 (50.0)	3 (37.5)	1 (12.5)	8 (100.0)
P value (Chi-square)	0.235			
rpoB gene				
Wild-type, no. (% within *rpoB*)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)
Mutated, no. (% within *rpoB*)	11 (57.9)	4 (21.1)	4 (21.1)	19 (100.0)
P value (Chi-square)	0.507			

*Statistically significant association

Macrophages incubated for several days in the presence of aminoglycosides accumulate these drugs inside their cytoplasm and were found to trigger anti-bactericidal activities of the macrophages through upregulation of killing mechanisms including reactive oxygen intermediates.[13] Accordingly, we suggest a mechanistic explanation for our result. Favourable response to second-line treatment arises because second-line agents, aminoglycosides (possibly other drugs), activate macrophages to upregulate oxidative killing mechanisms at the site *M. tuberculosis* infection. Strains with a *katG315*-mutation are sensitive to 'oxidative killing' owing to diminished catalase activity thereby clearing infection. The *katG315* mutants are sensitive to resulting in killing of bacteria by 'oxidative killing'.

Conclusion

In conclusion, these preliminary data implicate *katG315* mutation as a potential prognostic for treatment response to second-line anti-TB drugs; however, it needs to be confirmed in a larger study.

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