Research Article

The Gene Mutation and Drug Resistant of *Mycobacterium tuberculosis* in Patients of Chongqing

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Received: 06 March 2018; **Accepted:** 09 March 2018; **Published:** 12 March 2018

Abstract

Objective: To detect the infection and drug resistance *of Mycobacterium tuberculosis* (MTB) in Chongqing and provide a scientific basis for the prevention and treatment of tuberculosis.

Methods: DNA was collected from all new suspected patients in Chongqing from January 2014 to September 2017, Genechip technology was used to identify Mtb strain. Genechip technology detects mutations in the ropB gene (associated with resistance to rifampicin) at locus 511, locus 513, locus 516 locus 526, locus 531 or locus 533. Genechip technology was also used to detect mutations in the KatG gene and inhA gene.

Results: Genechip revealed that the Mtb infected male accounted for 73.98% and female accounted for 26.12%.The total drug resistance rate of rifampicin and isoniazid were 11.2% (122/110771). Genechip revealed that ropB gene of 72 strains were mutation. The highest mutation site was 531 (TCG) locus (37.5%, 27/72). The people with katG and inhA gene mutation were 50 patients. The most common mutation site was 315 (AGC) locus. The cavity, history of treatment, and irregular medication were the risk factor of drug-resistant Mtb.

Conclusion: Our report demonstrated the infected ratio and the drug resistant types of Mtb in Chongqing district. We should strengthen health management and provide psychosocial support, in order to reduce the risk of drug-resistant Mtb

Keywords: *Mycobacterium tuberculosis*; Gene mutation; Drug resistant

1. Introduction

Cancer-associated retinopathy (CAR) is a challenging clinical entity with often delayed diagnosis and difficult prognosis. The condition occurs in patients with typically known systemic malignancy but can precede cancer diagnosis. The clinical phenotype is varied, but typically includes optic nerve pallor, retinal vascular attenuation and visual field loss in the absence of obvious peripheral retinal abnormalities. Diagnosis is typically confirmed by serologic testing for anti-retinal antibodies. Numerous treatment options including systemic immune suppression with intravenous corticosteroids, intravenous immunoglobulins (IV Ig), and plasmapheresis have been used with mixed results. Even with treatment of the systemic malignancy, the prognosis typically involves worsening visual field loss. Anecdotal reports of intravitreal (intraocular) steroid injections have been able to demonstrate stability of visual field loss. This case report details the course of a patient with serologically confirmed cancer-associated retinopathy who showed initial improvement and later stabilization of visual acuity, optic nerve structure and function after a single intravitreal steroid injection in one eye.

2. Materials and Methods

2.1 Research population

From January 2014 to September 2017, the sputum, urine, pleural effusion, cerebrospinal fluid, and puncture fluid samples were collected from 6557 suspected TB patients (18-75 years) in The First Affiliated Hospital of Chongqing Medical University. All the patients were negative for hepatitis B virus, hepatitis C virus, human immunodeficiency virus (HIV),combined tumor and other symptoms of liver damage. This study was approved by the Institutional Review Board (IRB) committee of The First Affiliated Hospital of Chongqing Medical University. Written consent given by the patients was waived by the approving IRB.

2.2 Detection by DNA microarray chip

This study was based on the designing of oligonucleotide probes which can specifically detect the specific gene site of Mtb, and the mutations on the promoter of rpoB, KatG and inhA. Briefly, the DNA microarray chip technique (20) was used to test mutations in the rpoB gene at the 511, 513, 526, 531 and 533 codons. Common mutation sites to give an indication of RFP resistance. For INH resistance, the katG315 and inhA-15 mutation sites were assessed. The Mtb population detection kit and GeeDom Mtb drug detection kits (CapitalBio Corporation, Beijing, China) were operated according to the instruction of manufactory. The nucleic acid was extracted and PCR amplification. Once combined with a hybridization buffer (CapitalBio Corporation), the products were placed in a BioMixer chip hybridization instrument (CapitalBio Corporation) for hybridization. Then products were put in a slidewasher 8 chip dry cleaning instrument (CapitalBio Corporation) for washing and drying.Finally, the chip was placed in chip identification system (CapitalBio Corporation) for scanning and interpretation (LuxScanTM 10K/B software, CapitalBio Corporation).

2.3 Statistical analysis

Mann-Whitney U tests of SSPS12.0 (SPSS, Inc., Chicago, IL, USA) were used to assess the difference between different groups. A two-tailed $P<0.05$ was considered statistically significant. The distribution of the study variables

was calculated using means with standard deviations for normal continuous variables or using median with quartile range for skewness variables, and frequencies and percent for categorical variables. For continuous variable comparisons, Student's t tests were used when equality of variances was satisfied, otherwise Satterthwaite-tests were conducted. We assessed effect of each "risk" factor for TB using binary logistic regression. Stepwise logistic regression was performed in this study.

3. Results

3.1 Mtb positive patients and positive types distribution

We detected 6557 samples from 2014 to 2017 using Mtb strain identification gene chip and the Mtb positive ratio was 16.89%. The positive ratio in 2014-2017 was 27.14%, 21.13%, 14.46% and 12.67%, respectively (Table 1). The sputum, urine, pleural effusion, cerebrospinal fluid and puncture fluid were include in the sample types, which accounted for 22.9%, 6.5%, 17.5%, 15.8% and 37.3% , respectively (Table 2).

Year	Total samples	Positive samples	Positive (%)
2014	943	256	27.14
2015	1203	254	21.13
2016	2123	307	14.46
2017	2288	290	12.67
2014-2017	6557	1107	16.89

Table 1: The positive sample in the 6557 patient of Chongqing

Sample type	Sputum	Urine	Pleural Effusion	Cerebrospinal Fluid	Puncture Fluid
Sample No.	254	72	194	175	412
Positive (%)	22.9	6.5	17.5	15.8	37.3

Table 2: The sample type distribution in 1107 Mtb positive patient

3.2 The distribution of Mtb infected and drug-resistant patient

We then detected the sensitive situation of REF and INH in the 1107 Mtb positive samples, using the drug sensitive identification gene chip. The Table 3 demonstrated that infected male accounted for 73.98% (819/1107), and female accounted for 26.12% ($P<0.05$). Less than 20 years group, 20-39 years group, 40-60 years group and >60 years

group accounted for 3.52% (39/1107, 18.33% (203/1107), 32.61% (361/1107), 45.52% (504/1107), respectively. Therefore, the Mtb infected ratio increased by age. The general ratio of INH-resistant and RFP-resistant was 11.02 % (122/1107). The INH-resistant and RFP-resistant ratio were 5.32% (59/1107) and 3.41% (38/1107), respectively. Both INH and RFP resistant ratio was 2.25 % (25/1107). According to the age group, the drug-resistant ratio of the >60 years group was the highest, accounting for 4.24% (47/1107).

Drug sensitive type	**Gender**		**Age groups (years)**			
	Male	**Female**	**<20**	**20-39**	**40-60**	**>60**
Both sensitive	744	241	36	176	316	457
Resistant-REF	27	11	1	7	16	14
Resistant-INH	33	26	1	13	21	24
Both resistant	15	10	1	7	8	9
Total	819	288	39	203	361	504

Table 3: The drug sensitive types in 1107 Mtb positive patient

3.3 The mutation site of ropB, katG and inhA gene

The Table 4 showed the people with ropB gene mutation were 72 patients. Of these, the patients with single mutation were 68, double mutations were 3 patients, and triple mutations were 2 patients. The highest mutation site was 531 (TCG) locus (37.5%, 27/72). The people with katG and inhA gene mutation were 50 patients. Of these, the katG and inhA gene mutation ratio were 44.0% (22/50) and 42% (21/50), respectively. Meanwhile, both catch and inhale gene mutation ratio was 14% (7/50). The most common mutation site was 315 (AGC) locus.

Gene	**Mutation site**	**Mutation type**	**Positive samples**	**Mutation rate (%)**
rpoB	511 (CTG)	T→C	11	9.02
	513 (CAA)	C→A, A→C	3	2.45
	516 (GAC)	A→T, A→G	7	5.74
	526 (CAG)	C→T, C→G, A→T, A→G	13	10.66
	531 (TCG)	C→T, C→G	27	22.13
	533 (CTG)	T→C	7	5.73
	526, 533		1	0.82
	526, 531		1	0.82
	511, 516, 531		1	0.82

	511, 516		1	0.82
katG	315 (AGC)	G→C, G→A	22	18.03
inhA	-15 (ACG)	C→T	21	17.21
katG, inhA	315, -15		7	5.73

Table 4: The distribution of ropB, inhale, katG gene mutation

3.4 The risk factor analysis of drug- resistant Mtb

The Table 5 demonstrated the age, marital status, cavity, history of treatment, and regular medication were statistically different between drug-resistant and control group (drug-sensitivity) ($P<0.05$). Otherwise, educational level and occupation have no difference between drug-resistant and control group (drug-sensitivity) ($P>0.05$).

Social demographic characteristics	**Case group (%) n=122**	**Control group (%) n=985**	**P**	**OR (95% CI)**
Age(year)				
<40	30	212	0.121	1.011 (0.720-1.430)
≥40	92	773	0.023	0.452 (0.312-0.897)
Marital status				
Unmarried/other	45	321	0.223	0.776 (0.579-1.324)
Married	77	664	0.012	0.440 (0.305-0.636)
Educational level				
Higher education	21	143	0.582	1.132 (0.728-1.760)
Secondary education or below	101	842	0.457	1.101 (0.643-1.574)
Occupation				
Staff	10	124	0.267	1.818 (0.857-3.859)
Housekeeping, domestic chores and unemployed	76	578	0.056	0.962 (0.460-2.011)
Worker	22	209	0.302	1.261 (0.574-2.772)

other	14	74	0.295	1.661 (0.783-3.292)
History of treatment				
Yes	51	671	0.023	1.394 (0.759-2.583)
No	71	314	0.507	1.952 (0.843-2.351)
Cavity				
Yes	42	269	0.031	1.467 (1.018-2.113)
No	80	716	0.418	1.699 (0.986-2.459)
Regular medication				
Yes	73	809	0.673	1.363 (0.824-2.087)
No	49	176	0.016	2.115 (1.225-3.195)

Table 5: Results of risk factors for MDR-TB by logistic regression analysis

4. Discussion

The spread of Mtb seriously affected the people in the world. Moreover, the drug-resistant Mtb and its co-infection with HIV have seriously affected TB prevention and treatment [11]. Inherently, this has meant deterioration in the control of epidemics.

RFP and INH are primarily first-line anti-TB drugs. However, the effectiveness of the drugs has been greatly affected by the increase in drug resistance. A previous study has also suggested that in TB clinical strains demonstrate high levels of RFP (13.3%), INH (24.6%), and multi-drug (10.5%) resistance [5].

The most of RFP resistance related gene mutations located in the rpoB gene. The mutations on the 531 Ser, 526 His and 516 Asp codons accounted for 85% of the strains resistant to drugs. INH is another first-line anti-TB drug that is used together with RFP. Most INH resistance related to gene mutation were identified in the katG315 and inhA-15 mutations [11]. The primary mutation mechanism of INH-resistance in the MTB katG gene investigated was 315 AGC→ACC, Ser→Thr (S315T).

We detected the suspected Mtb patients in Chongqing using gene chip method and the positive ration was 16.89 %. The general ratio of INH-resistant and RFP-resistant was 11.02 %,which was less than 13.49% in the report of Pang Y et al. [13]. The reason might be that we recruited new patients not retreatment patients. The Mtb infected male

accounted for 73.98% and female accounted for 26.12%.The most Mtb infected people were men and the ratio of infected men/women was 2.5. The data showed no difference with the report of Pang Y et al. [13]. Our report demonstrated the history of treatment was the risk factor of the drug-resistant , which consisted with the report of the report of Lomtadze N et al. [14]. Moreover, irregular medication was another risk factor, which consisted with the report of the report of DIANDÉ S et al. [15]. The irregular medication caused disease relapse and drug-resistant Mtb became the dominant bacteria. The cavity was also another risk factor in our report, which In accordance with the study of Ahmad AM et al. [16]. There were lots of drug-resistant Mtb in the cavity, so the Mtb might spread easily.

Our report demonstrated the infected ratio and the drug resistant types of Mtb in Chongqing district. The risk factors associated with drug resistant are complex; we should reinforce early detection, rapid diagnosis, and standardize therapy, in order to make sure that the patients take the full course of the treatment to reduce the risk of drug resistant types of Mtb.

Acknowledgements

This work was supported by a grant from National Natural Science Foundation of China (No 81501818 of Yishu Tang) and the National Key Clinical Specialties Construction Program of China.

Conflict of interest

The authors declare no conflicts of interest.

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Citation: Yishu Tang, Peiyang Song, Huiting Su.The Gene Mutation and Drug Resistant of *Mycobacterium tuberculosis* in Patients of Chongqing. Archives of Microbiology & Immunology 2 (2018): 010-017.