Genotype Diversity of Isolates of Silicotuberculosis and Pulmonary Tuberculosis in a Certain Mining Area

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Pneumoconiosis and pulmonary tuberculosis seriously affect the health of coal miners. Tuberculosis complicating silicosis is often called silicotuberculosis. The purpose of this research is to explore whether silicotuberculosis isolates have independent clustering characteristics or not. We isolated 42 strains of *Mycobacterium tuberculosis* from tuberculosis patients in a certain mining area, among which 16 were silicotuberculosis strains and 26 were pulmonary tuberculosis strains. The polymorphism typing results of 12 mycobacterial interspersed repetitive-unit-variable-number tandem-repeat (MIRU-VNTR) loci showed that the gene polymorphism of the silicotuberculosis and pulmonary tuberculosis isolates at 26 and 40 MIRU loci was higher. The cluster analysis showed that the isolates had different cluster characteristics, indicating that *Mycobacterium tuberculosis* which is prevalent in pneumoconiosis patients may have independent clustering characteristics.

Key words: Pneumoconiosis, Tuberculosis, Silicotuberculosis, *Mycobacterium tuberculosis*, Coal miner.

Pneumoconiosis and pulmonary tuberculosis (PTB) are two different diseases that are closely related. Clinically, the disease resulting from the synergic action of silicious dust and *Mycobacterium tuberculosis* on workers exposed to silica is called silicotuberculosis. The first complete record of silicotuberculosis dates back to 1925 in South Africa, at which time *M. tuberculosis* was found during the postmortem of pneumoconiosis patients[1]. The high risk of mycobacterial infection in silica-exposed coal miners has elicited increasing attention, with emphasis on control[2]. The risk of a patient with silicosis developing tuberculosis is higher (2.8 to 39 times higher, depending on the severity of the silicosis) than that found for healthy controls[3]. PTB rates can be extremely high in silica-exposed groups with high background population TB and HIV rates, as is the case in South African gold miners with PTB rates of 3085 per 100 000 [4]. The incidence of silicotuberculosis is related to the severity of pneumoconiosis in the same mining area. However, the incidence of silicotuberculosis in different mining areas is not proportionate to the severity of pneumoconiosis among the coal workers[5]. Whether the incidence of pneumoconiosis is related to the type and pathogenicity of *M. tuberculosis* isolates of different mining areas, as well as to the influence of different prevalent rates of local PTB, remains unknown. Should the answer be "yes", silicotuberculosis isolates have independent clustering characteristics and are not worthy of in-depth investigation.

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China is a large coal-producing country; it is also one of the countries suffering from the most severe incidents of silicotuberculosis. The average prevalence rate of silicotuberculosis is 19.4%, which is 28 times higher than the average prevalence rate of internal pulmonary tuberculosis (0.7%)[6]. A silicotuberculosis patient can easily be a potential reservoir of infection to pneumoconiosis patients and to a healthy population. Therefore, the development of a typing study of *M. tuberculosis* in mining areas has an important implication for the prevention and control of silicotuberculosis. It is of interest to understand genotypic patterns of *M. tuberculosis* isolates in mining areas and how they compare to those of silicotuberculosis and PTB. Moreover, from a TB-control point of view, it is relevant to understand whether specific genotype families are over-represented among silica-exposed coal miners.

MATERIALS AND METHODS

Diagnosis of cases

All subjects were come from the department of pneumoconiosis and tuberculosis in the miner hospital. The diagnosis of silicosis was carried out by a group of 5-7 memberships on the basis of a history of exposure to silica, the characteristic radiological features and a clinical profile consisitent with the disease[7]. Silicotuberculosis patirnts were diagnosed by sputum smear microscopy and sputum culture based on pneumoconiosis. The relative epidemiological and clinical data of patients were collected including aged, residence, length of service, drug resistance rate, pneumoconiosis stage, etc. The study was approved by the ethical committee of Hebei United University (Document code: 10-006). All participating subjects signed the informed consent forms.

Strain isolation and identification

Forty-two strains of *M. tuberculosis* were isolated from male tuberculosis patients and were identified as human-like *M. tuberculosis* through acid-fast stain examination and *para-nitro-benzoic* acid and thiophene-2 carboxylic acid hydrazide culture media. Among these 42 strains, 16 were from silicotuberculosis patients and 26 were from PTB patients in the mining area. No kinship existed among the patients.

Mycobacterial interspersed repetitive-unit-variable-number tandem-repeat (MIRU-VNTR) polymorphism analysis

The MIRU-VNTR genotyping tool was established by Supply et al. for typing purposes [8,9]. The polymorphism of 12 MIRU-VNTR loci (2, 4, 10, 16, 20, 23, 24, 26, 27, 31, 39, and 40) tandem duplication units was confirmed using PCR products in the corresponding loci of the *M. tuberculosis* standard strain H37Rv used as control. Those 12 independent loci could identify almost strains of *M. tuberculosis* which were clustered by the IS6110 standard method[10], so we selected these 12 independent loci for typing the genotypes of *M. tuberculosis* strains. The Hunter-Gaston discriminative Index (HGI) value was used to show the resolution and the genetic polymorphism. The higher of HGI value, the discriminatory power of genotyping was better. Meanwhile, the MIRU loci of 10% of the strains were extracted for gene sequencing and for verification of the accuracy of the typing result. BioNumerics 3.0 cluster analysis software was used to divide clinical isolates into clustering and non-clustering. Clustering strains were divided into seven types according to the Cluster Cutoff value.

RESULTS

Demographics and clinical data of subjects

The data was collected on TB patients from inhabitant residing in the mining area in northern China, with a history of nearly a century, covers an area of 24.5 square kilometers, a population of 191 400 people. Epidemiologic data were available for 42 patients. They were the Han nationality and secondary pulmonary tuberculosis. Among them, the average age of patients was 71.75±3.89 in silicotuberculosis group, 49.08±19.25 in PTB group, with a statistical difference between two groups. Acording to the stage of 16 cases of pneumoconiosis, 4 cases were stage I; 10 cases in stage II; 2 cases in stage III. Accu mulated dust exposure time was 26.50 +/- 7.29 years.

In 42 strains of *Mycobacterium tuberculosis*, the sensitive strains to anti-tuberculosis drug were of 25 strains, accounting for 59.52%, and the resistant strain 17 strains, accounting for 40.48%. The resistant rate was higher in silicotuberculosis group than that in

simple tuberculosis group.

Gene polymorphism analysis of silicotuberculosis and PTB isolates

Among the 12 MIRU-VNTR polymorphic loci in the 42 strains of *M. tuberculosis*, MIRU-VNTR loci 26 and 40 of silicotuberculosis patients had higher polymorphism ($h > 0.6$) and loci 10, 31, and 39 had medium polymorphism ($0.3 \leq h < 0.6$). Loci 26, 31, and 40 of PTB patients had higher polymorphism and loci 10, 27, and 39 had medium polymorphism. The distribution difference in the gene polymorphic loci between the two groups of isolates was not statistical significant.

Cluster analysis of silicotuberculosis and PTB isolate genotype

The cluster analysis showed that the 42 strains of *M. tuberculosis* were divided into 7 clusters. "Cluster" referred to a group of *M. tuberculosis* with the same or similar DNA fingerprint features. Silicotuberculosis isolates were mostly gathered in Cluster II, III and VI. PTB isolates were gathered in Clusters II, VI and VII. Obvious difference in gene clustering distribution was observed between silicotuberculosis and PTB isolates (Fisher's exact test, *P*=0.014) (Table 1).

Table 1. Cluster analysis of 42 strains of *M. tuberculosis* isolate

Genotype cluster	No. of strains	No. of strains of silicotuberculosis	No. of strains of pulmonary tuberculosis
No clustering	7	2	9
With clustering	35	14	17
I	2	0	2
II	7	3	4
III	6	5	1
IV	3	1	2
V	3	2	1
VI	7	3	4
VII	3	0	3
Total	42	16	26

DISCUSSION

Molecular epidemiology research has found that non-associated strains in epidemiology generate unique genealogy characteristics, and that genealogies of associated strains are completely the same or similar. This is the first molecular epidemiologic study of comparing silicotuberculosis and PTB isolates in mining areas. The aim of this study was to acquire data that can prove whether an independent epidemic strain in silicotuberculosis patients exists. Based on MIRU-VNTR, our data showed that the distribution of the MIRU polymorphic loci of the silicotuberculosis group was similar to that of the PTB group, that is, higher polymorphism was observed at 26 and 40 loci. However, the research result was different from the distribution of MIRU polymorphic loci in domestic and foreign reports[9,11-12]. This difference may be ascribed to the varied *M. tuberculosis* isolates from different areas. Moreover, the number of isolates collected in the present survey was lesser compared with that of other studies. No clear difference was observed in the distribution of gene polymorphism between the isolates.

It is very interesting that, despite the same living environment background, silicotuberculosis isolates and PTB isolates showed different clustering characteristics. The former were gathered in type II, III and VI, whereas the latter were mostly gathered in types II, VI and VII. The clustering indicated the presence of an independent epidemic strain of *M. tuberculosis* in silicotuberculosis and PTB patients within the same mining area. One possible explanation for this difference could be the special exposure surroundings. Perhaps superiority TB strains are resided in the working environments of coal mine, but this remains to be determined.

Owing to its high resolution, the MIRU-VNTR technique is particularly useful for typing studies of *M. tuberculosis* in Asian countries[10, 13]. Under the premise of using the MIRU-VNTR method, the typing result of the tuberculosis isolates of the two groups of patients is therefore credible. Irrational antibiotic use of coal miners with pneumoconiosis and their delayed medical treatment after concurrent tuberculosis infection will cause less isolates. Thus, the conditions of the silicotuberculosis bacterial type in this mining area may not be completely reflected, and cannot fully embody the difference between silicotuberculosis and PTB. In future research, tuberculosis strains of silicotuberculosis and PTB patients in the coal mining areas should be collected to establish a strain library and genotype database of these two kinds of tuberculosis patients for systematic comparative analysis.

A major limitation of this study was that we did not have enough epidemiological information available to verify the transmission links indicated by typing method. It was therefore, not possible to ascertain the validity of epidemiological links indicated.

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