Original Article

Mycobacterium tuberculosis Genotypes in patients developing pulmonary tuberculosis related acute respiratory distress syndrome

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Abstract

Background:

Acute Respiratory Distress Syndrome is an uncommon but frequently fatal presentation of pulmonary TB. We attempted to identify whether a specific M. tuberculosis genotype occurs more commonly in patients who develop ARDS.

Methods:

An observational study carried out at the Aga Khan University Hospital, Karachi, Pakistan enrolled all ARDS patients with tuberculosis. MTB isolates were spoligotyped for strain identification.

Results:

725 patients were admitted with pulmonary tuberculosis during the study period. Only 2.5% (18 patients) developed ARDS. Inpatient mortality rate was 58% (7 patients). Genotypes of M. tuberculosis isolates were predominantly CAS I (58.3%) and Unique strains (25%). Beijing and CAS subfamilies were less common; with each genotype identified in 8.3% patients respectively. All except one of the isolated strains were sensitive to usual first line anti-tubercular drugs.

Conclusion:

Our results indicate that the CAS1 strains are the most common genotypic strains causing severe respiratory disease in patients with ARDS.

Introduction

Tuberculosis (TB) remains a significant, global health problem, presenting to medical professionals from differing specialties and at varying levels of care. According to the World Health Organization, there are currently an estimated 14.4 million prevalent cases of TB worldwide [1], with an estimated 136 new cases per 100,000 populations and a mortality rate of 24 per 100,000 patients per year [2].

Pulmonary tuberculosis in immune competent individuals usually presents as a chronic respiratory illness with fevers and weight loss. Less frequently patients present with a rapidly progressive, acute respiratory failure which has a high risk of mortality (22 – 69%) [3-10]. Investigators have described a number of host factors; HLA- DR phenotypes [11,12], hematological, biochemical derangements and durations of disease that predict a greater risk of severe respiratory disease[7,11]. Studies on animal and macrophage models have demonstrated Mycobacterium tuberculosis strain specific effects on virulence and immunogenicity [13,14,15,16,17,18] . This suggests that genetic variability in M. tuberculosis may have important effects on phenotypic expression or may help explain the differences in clinical presentations.

We hypothesized that some M. tuberculosis strains may have a greater propensity to cause severe pulmonary disease, i.e. Acute Respiratory Distress Syndrome (ARDS). The objective of this study was to identify genotypic lineages of M. tuberculosis isolated from patients who developed ARDS from pulmonary tuberculosis.

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Materials and methods

Patients

In an observational, descriptive design, consecutive patients admitted to the hospital from January 2004 to March 2008 and who subsequently developed ARDS due to pulmonary tuberculosis were identified through disease – codes. ARDS was confirmed according to the American-European Consensus Conference [19] definitions. The diagnosis of tuberculosis was made after the growth of M. tuberculosis on culture of respiratory secretions (tracheal aspirates, bronchial lavage or expectorated sputum).

Methods

Clinical data were obtained from medical records and categorized as; demographics (age, sex, source of admission, co morbidities, APACHE score, pre-existing TB disease), time to diagnosis of TB, treatment (steroids, antituberculous therapy) and outcomes (mortality rate, sensitivity patterns, strain typing of isolates).

Microbial culture & Strain identification

Stored M.tuberculosis strains from the selected patients were prospectively re-cultured. The strains had originally been isolated from specimen cultured using BACTEC and identified on the basis of NAP and Niacin positivity and stored at -80oC [20,21,22]. The strains were recultured using Middlebrook agar and the isolates spoligotyped for strain identification. DNA extracted from M. tuberculosis isolates of ARDS patients was carried out by the cetyltrimethylammonium bromide method (CTAB) [23]. Spoligotyping based on 43 spacers of the direct repeat region of M. tuberculosis complex was carried out with primers DRa (5' GGTTTTGGGTCTGACGAC 3') and DRb (5' CCGAGAGGGGACGGAAAC 3') as described by Kamerbeek et al [24]. All isolates are spoligotyped and analyzed with the Bionumerics software programme. Dendrogram was generated using unweighted-pair-group method with arithmetic averages (UPGMA) calculations. The typing method is based on DNA polymorphism present at one particular chromosomal locus, the 'Direct Repeat' region, which is uniquely present in Mycobacterium tuberculosis complex bacteria. With this method the presence or absence in the DR region of 43 spacers of known sequence can be detected by hybridization of PCR-amplified spacer DNA to oligonucleotides complementary to spacer sequences.

Statistical Analysis

Categorical variables are presented as proportions; continuous variables are reported as means with standard deviations.

The study design was approved by the hospital Ethical Review Committee.

Results

From 2004 – 2008, 725 patients with pulmonary tuberculosis were admitted to the hospital; 18 patients developed ARDS. Strains from 12 patients were successfully recultured and genotyped.

The average age of patients was 54.6 ±16.3 years, with males tending to predominate; 66.7% (8 patients). Severity of illness measured by APACHE II scores at admission had a mean value of 17.4 ±2 (range 15-21). Approximately 50% (6) patients had a remote history of Pulmonary TB. Most patients were admitted from the emergency room; 91.6% (11 patients). All related a short history of progressive respiratory illness, mean 9 days (range 4 - 14 days. Most patients developed ARDS after hospitalization; 66% (8 patients) on an average of 1.14 days (range 1-7 days) after admission to the hospital. 33% (4 patients) had been started on 4 drug (INH, Rifampicin, Ethambutol, Pyrazinamide) antituberculous therapy by their primary physicians prior to hospitalization. HIV status assessment is not a routine practice and therefore was not available for these patients.

All patients were treated with weight - based dosages of 4 first line antituberculous drugs; INH, Rifampicin, Ethambutol & Pyrazinamide. 41.6% (5 patients) were additionally treated with intravenous corticosteroids in doses of 1 mg / kg/day. Inpatient mortality rate was 58% (7 patients), whilst 42 % (5 patients) survived

to hospital discharge. Mean duration of hospitalization was 11.8 days ± 12.2 (range 1-50).

The most common genotypic strain identified was CAS I, in 58.3% patients, Unique strains were isolated in 25% patients, followed by the Beijing strain and CAS Sub families in 8.3% patients each.

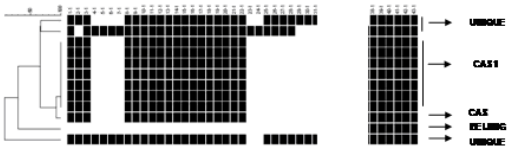

Figure 1: Dendrogram of M.tuberculosis from patients with Acute Respiratory Distress Syndrome. All isolates were spoligotyped and analyzed with the Bionumerics software programme. Dendrogram was generated using unweighted-pair-group method with arithmetic averages (UPGMA) calculations. The clustering pattern is illustrated as identified by clusters in SpolDB4.0.

A single patient was infected with a multidrug resistant (resistance to INH, Rifampicin and Ethambutol) M. tuberculosis strain. This strain belonged to the CAS I family. All other isolates demonstrated in vitro sensitivity to first line antituberculer medications.

Discussion

Genotyping of M.tuberculosis strains causing TB with severe respiratory disease (ARDS) revealed a predominance of the CAS 1 strain with lesser proportions of other strains in our study.

Molecular genotyping has identified the existence of a number of M.tuberculosis strains worldwide. Using typing techniques, investigators have determined that M.tuberculosis lineages tend to be geographically and ethnically restricted. The East African Indian (EAI), Central Asian Strain (CAS) and Beijing families have been identified with greatest frequency from Asia, with the Central Asian strain 1 type (CAS 1) or Delhi type genogroup occurring commonly in India and Pakistan [25,26,27]. Hasan et al [28] reported that of 926 M. tuberculosis strains studied from isolates sent to a central laboratory in Pakistan,, 721(78%) were grouped into 59 "shared types", while 205 (22%) were identified as "Orphan" spoligotypes. Amongst the predominant genotypes 61% were Central Asian strains (CAS ; including CAS1, CAS sub-families and Orphan Pak clusters), 4% East African-Indian (EAI), 3% Beijing, 2% poorly defined TB strains (T), 2% Haarlem and LAM (0.2).

ARDS is an extremely severe form of lung injury caused by a number of infectious and noninfectious insults. TB causing this severity of lung injury is an infrequent presentation, but when it occurs it carries a high morbidity and mortality. Host factors such as greater than 30 days duration of illness, disseminated disease (military TB), absolute lymphocyte counts < 1625/mm3 and serum alanine transferase levels > 100 IU/L predict a greater likelihood of developing ARDS after pulmonary M. tuberculosis infection [25]. Once ARDS develops, delays in diagnosis and treatment6, bilateral consolidations, multiorgan failure [3], disseminated disease [7,8], oxygenation index < 108 and APACHE II scores > 18 [7,10], are associated with a higher mortality.

Whether genotypic lineages affects phenotypic expression of disease has not been previously described for ARDS. An extensive MEDLINE search for any reports of associations between phenotypic expression and genotype revealed only a handful of studies. In 2001, Van Crevel and associates [29] reported that the Beijing strain may have specific pathogenic abilities i.e. fevers unrelated to the disease severity, drug resistance or toxicity. Since then, the Beijing lineage has been shown to dominate the TB epidemic in East Asia and been associated with hypervirulence, high rates of transmission and drug resistance [30]. Interestingly in our results, we found a lesser association of the Beijing genotype with the patients who developed ARDS. Thwaites and colleagues [31] reported that those infected with the Euro- American genotype had a greater incidence of consolidations on chest radiograph (p 0.006) compared to those infected with other genotypes. In a separate publication [32], the authors reported their discovery of a protective association between the Euro-

American lineage and disseminated disease (OR 0.39, 0.19 - 0.8). Lazzarini and coworkers [33] studied the RD (Rio) M. tuberculosis strain, which is a Latin American-Mediterranean sublineage that is the predominant cause of TB in Rio de Janeiro, Brazil. Multivariate analysis found a significant association between TB caused by RD (Rio) strains and pulmonary cavitation. In our study mycobacterial strains spoligotyping revealed a predominance of CAS 1 strains in patients with ARDS. This may suggest that this lineage may be associated with greater severity of lung disease either through direct damage from aggressive mycobacterial replication or through the effects of a magnified host response. We also found that infection with the Beijing lineage resulted in less severe forms of lung injury. Patients who tended to have milder forms of pulmonary disease tended to have a more diverse representation on spoligotyping with an almost equal distribution of isolates infected with clustered genotypes (CAS 1, CAS subfamily and Beijing lineages) and unique genotypes.

A significant limitation of our study is the small numbers of patients with ARDS and thereby the small numbers of isolates we were able to spoligotype. Only 2.4% of all tuberculosis patients presenting to our hospital had ARDS. However, ARDS due to pulmonary TB is a rare phenomenon and our low incidence rate is comparable to results presented by Sharma et al [7], who reported a 1% incidence of ARDS amongst all patients presenting with pulmonary TB in countries where this disease is endemic. Ideally larger numbers of patients from multiple centers and across countries would be needed to make a definitive association between genotypes and disease expression.

Conclusion:

Our results suggest that the strains associated with severe lung injury such as ARDS appear to predominantly belong to the mycobacterial genotype CAS 1. More interestingly, the Beijing strain, which has been associated with hypervirulence, was less significant in this group of patients with severe lung disease. These findings would suggest that genotypes may have some influence on disease severity.

Acknowledgements: None

Sources of funding: None

Conflict of interest: The authors declare no conflict of interest.

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