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Original article

Clinical evaluation of high mobility group box 1 protein in *Legionella pneumophila* pneumonia

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ABSTRACT

High mobility group box 1 (HMGB-1) protein is involved in acute lung injury due to various etiologies. We evaluated HMGB-1 levels in sera and bronchoalveolar fluids in patients with pneumonia caused by *Legionella pneumophila*. Levels of HMGB-1 in the sera of patients with *L. pneumophila* pneumonia (32 cases) and control subjects (24 cases) were determined. Serum HMGB-1 levels in *Legionella* pneumonia were similar to those of the control subjects. No significant correlation between HMGB-1 levels and other biomarkers and the outcome of cases was observed. In contrast, HMGB-1 levels, as well as interferon-γ, in bronchoalveolar (BA) fluids from severe *L. pneumophila* pneumonia (7 cases) were significantly higher than those in the sera of identical patients. HMGB-1 levels in BA fluids were relatively higher in pneumonia cases with ALI than those without ALI. Our findings suggest that intra-pulmonary HMGB-1 may be involved in the pathophysiology of pneumonia caused by *L. pneumophila*.

1. Introduction

Bacteria of the genus *Legionella* are important causative agents of epidemic and sporadic pneumonia in humans [1]. The most common species in the genus is *Legionella pneumophila*. *Legionella* species is an important pathogen causing severe community-acquired and hospital-acquired pneumonia requiring intensive care unit admission [2–4]. *Legionella* pneumonia progresses rapidly and is often complicated by acute lung injury/acute respiratory distress syndrome (ALI/ARDS) [5,6].

The high mobility group box 1 (HMGB-1) protein belongs to the nuclear nonhistone protein family, which is involved the transcription of genes within the nucleus [7]. Recent studies have shown that the HMGB-1 protein is released from activated macrophages and dying cells. The released extracellular HMGB-1 works as an inflammatory cytokine and is involved in the late phase of sepsis [8] and ALI/ARDS [9]. HMGB-1 was also released from alveolar epithelial cells and alveolar macrophages when they were infected with *L. pneumophila* [10,11], suggesting that this cytokine is involved in the pathophysiology of *Legionella* pneumonia. However, the clinical significance of HMGB-1 in the disease remains unclear.

In this study, we examined the concentrations of HMGB-1 in the sera and bronchoalveolar (BA) fluids of patients with *L. pneumophila* pneumonia to evaluate the clinical role of HMGB-1 in *Legionella* pneumonia.

2. Materials and methods

2.1. Study population

Thirty-six consecutive cases with *L. pneumophila* pneumonia diagnosed in our laboratory from 1997 through 2007 were included in this study. Four cases were excluded because of a lack of appropriate samples. Blood samples were obtained for the conventional clinical diagnosis of the patients. BA fluids were obtained with written informed consent when required to diagnose *Legionella* pneumonia. The samples were collected when the diagnosis of *Legionella* pneumonia was established. These samples were stored at −80 °C until further use. Medical chart reviews were used to obtain information regarding the laboratory findings and clinical outcome for each patient. Severity of pneumonia was determined using pneumonia severity index [12].

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This study was approved by the University of the Ryukyus Institutional Review Board. The need for informed consent from each patient for inclusion in this study was waived because this study was retrospective in approach, which caused no additional adverse events in any subject.

2.2. Diagnosis of Legionella pneumonia

Pneumonia was diagnosed based on clinical presentation (symptoms and physical examination), chest X-ray findings, and laboratory data. The diagnosis of *Legionella* pneumonia was confirmed by detecting *Legionella* in culture, the elevation of antibody titers in paired sera, and/or the detection of its specific antigen in the urine. Control subjects had no known infectious disease, liver disease, or kidney disease.

2.3. Determination of HMGB-1 and other biomarkers

The HMGB-1 concentration in each sample was determined via a sandwich ELISA (Shino-Test Co., Tokyo, Japan) using recombinant swine HMGB-1 as a standard [13]. The detection limit of HMGB-1 was 0.1 ng/mL. Hepatocyte growth factor (HGF) and interferon-gamma (IFN-γ) levels in each sample were determined using a sandwich ELISA (R&D Systems, Minneapolis, MN, USA). The lowest detection limits for HGF and IFN-γ were 40 pg/mL and 8 pg/mL, respectively. The albumin concentration in each sample was determined using a QuantiChrom BCG albumin assay kit (BioAssay Systems, Hayward, CA, USA).

3. Statistical analysis

Differences between groups were analyzed using the unpaired *t*-test, Fischer's exact probability test, the Mann–Whitney *U* test, or the Wilcoxon matched-pairs signed rank test using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA). The association between biomarkers was evaluated by Pearson's correlation coefficient using the IBM SPSS statistics version 21 (SPSS, Inc., Chicago, IL, USA).

4. Results

The average age of the 32 patients with *Legionella* pneumonia was 59.8 ± 11.1 years; this group included 28 men and 4 women. The average of age of the 24 control subjects was 54.2 ± 19.1 years. These subjects included 17 men and 7 women. Statistical analyses of age (unpaired *t*-test) and sex (Fischer's exact probability test) between groups showed no significant differences. The serum HMGB-1 concentrations for patients and control subjects were determined. The serum HMGB-1 levels of the *L. pneumophila* pneumonia patients (mean ± SD; 5.8 ± 6.4 ng/mL) were similar to those of the healthy control subjects (mean ± SD; 4.6 ± 3.2 ng/mL) (Fig. 1). No significant linear associations between serum HMGB-1 and other biomarkers (white blood cell counts, C-reactive protein, lactate dehydrogenase) and oxygenation parameters (PaO_2 and PaO_2/FiO_2) were observed (Table 1). Additionally, Serum HMGB-1 levels did neither differ between pneumonia cases with different pneumonia severity index [12] (Table 2) nor between 24 survivors (mean ± SD; 5.8 ± 6.8 ng/mL) and 8 non-survivors (mean ± SD; 5.6 ± 5.0 ng/mL).

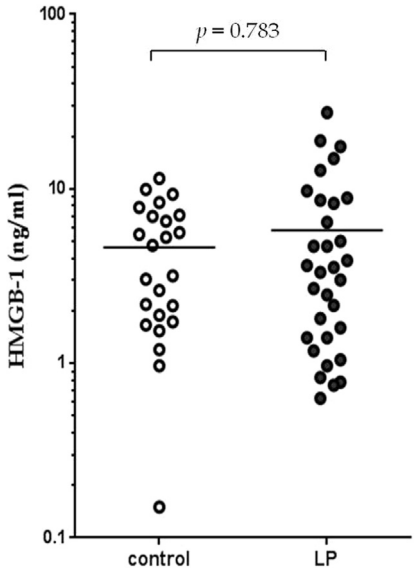

Fig. 1. Serum concentration of HMGB-1 in patients with *Legionella pneumophila* pneumonia and healthy control subjects. HMGB-1 levels were determined using a sandwich ELISA. No significant difference was observed between the 2 groups (Mann–Whitney *U* test).

Next, we determined the HMGB-1 levels in the BA fluids of 7 patients with *Legionella* pneumonia. The HMGB-1 levels in the BA fluids were significantly higher than those in the sera of identical patients (Fig. 2a). The HMGB-1 levels in the BA fluids of patients with ALI (PaO_2/FiO_2 ratio ≤ 200) were relatively higher than those in patients without ALI (PaO_2/FiO_2 ratio > 200) (Fig. 2b). Similar results were observed when HMGB-1 concentrations were normalized using albumin concentration (data not shown). IFN-γ concentrations were also higher in BA fluids than in sera of identical patients, while HGF concentrations were similar in sera and BA fluids (Fig. 2c and d).

5. Discussion

This study revealed that serum HMGB-1 levels of *Legionella* pneumonia were similar to those in healthy control subjects. This finding disagrees with the results of previous studies in which serum/plasma HMGB-1 was higher in community-acquired infections and pneumonia than in healthy subjects [14–16]. In these reports, the average serum HMGB-1 in control subjects was lower

Table 1
Association between serum cytokine concentrations and various serum biomarkers and the oxygenation index.

	Pearson's correlation coefficient (*p* value)				
	WBC	LDH	CRP	PaO_2	PaO_2/FiO_2
HMGB-1	−0.021 (0.911)	0.089 (0.641)	0.309 (0.103)	0.045 (0.813)	0.118 (0.535)
HGF	−0.149 (0.393)	0.517 (0.002)*	0.058 (0.753)	−0.066 (0.717)	−0.077 (0.669)
IFN-γ	−0.176 (0.370)	0.012 (0.952)	0.036 (0.864)	−0.080 (0.944)	0.346 (0.077)

HMGB-1: high mobility group box 1 protein, HGF: hepatocyte growth factor, IFN-γ: interferon-gamma, WBC: white blood cell counts, LDH: lactate dehydrogenase, CRP: C-reactive protein, PaO_2: Arterial oxygen pressure.
Asterisks represent significant associations.