

The relationship between serum endothelin (ET)-1 and wheezing status in the children with *Mycoplasma pneumoniae* pneumonia

Chung HL, Kim SG, Shin IH. The relationship between serum endothelin (ET)-1 and wheezing status in the children with *Mycoplasma pneumoniae* pneumonia.

Pediatr Allergy Immunol 2006; 17: 285–290.

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Although *Mycoplasma pneumoniae* infection is known to be associated with acute wheezing and the exacerbation of asthma, the mechanism by which this pathogen contributes to the development of wheeze-related symptoms is not fully understood. The aim of our study was to examine serum endothelin (ET)-1 and other cytokines in acute *M. pneumoniae* pneumonia and investigate if there is any relation between these inflammatory mediators and the occurrence of wheezing. We studied 53 patients, aged 3–13 yr, who admitted with pneumonia. These patients were divided into three groups: *M. pneumoniae* pneumonia with wheeze ($n = 23$) and without wheeze ($n = 19$), and the patients without the evidence of *M. pneumoniae* infection ($n = 11$). Age-matched controls ($n = 10$) were also studied. The serum concentrations of ET-1, interleukin (IL)-5 and IL-18 were measured using ELISA kits in patient groups and controls. The patients with *M. pneumoniae* pneumonia had significantly higher serum ET-1 than those without evidence of *M. pneumoniae* infection. In the presence of *M. pneumoniae* pneumonia, ET-1 concentrations were significantly higher in the patients with wheeze than those without wheeze. IL-5 and IL-18 in each patient group were higher compared to controls. However, no significant between-group difference was observed. Total serum IgE levels were significantly higher in the patients with *M. pneumoniae* pneumonia and wheeze than in those without wheeze. A positive correlation was observed between serum ET-1 and total IgE in the patients with *M. pneumoniae* pneumonia and wheeze. Our results may suggest a role of ET-1 in the occurrence of acute wheezing or exacerbation of asthma associated with *M. pneumoniae* pneumonia.

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Key words: *M. pneumoniae* pneumonia; ET-1

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Accepted 11 January 2006

Mycoplasma pneumoniae is a frequent pathogen that is responsible for the lower respiratory tract infections in children. Acute wheezing associated with increased bronchoconstriction and impaired pulmonary function, which lasts for long time after the initial infection have been described (1–4). Acute *M. pneumoniae* infection has been diagnosed in up to 20%–25% of children experiencing asthma exacerbation (5, 6). Although the clinical significance of *M. pneumoniae* is becoming

evident, the pathophysiological mechanisms by which the pathogen contributes to the development of wheeze-related symptoms and exacerbation of asthma are not yet fully understood.

Endothelin (ET)-1 is a 21 amino acid peptide released from vascular endothelial cells and bronchial epithelial cells (7). ET-1 is a potent mediator of smooth muscle contraction and bronchoconstriction also is known to stimulate the secretion of mucus and activate the

inflammatory cells (8–10). There was a study, which showed the elevation of ET-1 biosynthesis in human endothelial cells with mycoplasma infection (11).

Interleukin (IL)-5 was reported to play a critical role in the development of airway hyper-responsiveness in a murine model of respiratory syncytial virus (RSV) infection (12). A recent study reported the association between increased serum IL-5 and wheeze-related symptoms in children with *M. pneumoniae* infection (13).

IL-18, in collaboration with IL-12, stimulates Th1 immune responses, and plays an important role in the host defense against infection (14). IL-18 was shown to increase in serum during acute *M. pneumoniae* pneumonia, which was related with the severity of disease (15).

In our study, we determined serum ET-1, IL-5 and IL-18 in the children with acute *M. pneumoniae* pneumonia and investigated the association between the increase of these inflammatory mediators and the occurrence of wheezing.

Materials and methods

Study subjects

Fifty-three patients, aged 3–13 yr, who were admitted with acute pneumonia from January 2003 through June 2004, were enrolled. All patients showed clinical and radiological evidence of pneumonia. The patients were divided into three groups: the patients who showed the laboratory evidence of *M. pneumoniae* pneumonia and wheeze (group 1, $n = 23$), the patients who had *M. pneumoniae* pneumonia without wheeze (group 2, $n = 19$), and the patients without the evidence of *M. pneumoniae* infection and without wheeze (group 3, $n = 11$). The patients who had been diagnosed to have asthma or other chronic pulmonary disease were excluded. None of the patients studied were receiving anti-inflammatory medications on admission. The patients who had hypoxia on admission (as defined by PaO_2 less than 85 mm Hg (16)) were excluded because hypoxia itself is an important stimulus for ET-1 production (17).

The control group ($n = 10$) included the children who were admitted for tonsillectomy. These control subjects had no respiratory symptoms on admission, no history of asthma, and no evidence of other infectious or inflammatory diseases.

Informed consents were obtained from all parents and this study was approved by the Catholic University of Taegu Institutional Review Board.

Laboratory methods

Serum samples for determination of antibodies to *M. pneumoniae* were obtained on admission and during convalescence period after 3–4 wk. The diagnosis of *M. pneumoniae* infection was made if the subject had a significant antibody response in serum defined as: specific IgG $> 1:320$ and positive specific IgM response on admission or a four-fold increase in IgG titer during convalescence and positive specific IgM response on admission. Specific IgG antibodies were determined using microparticle agglutination assay (MAG) (Serodia-Myco II, Fujirebio, Tokyo, Japan), a more advanced hemagglutination test (18). Specific IgM antibodies were determined using ELISA kit (Euroimmune, Lubeck, Germany) and the values above the reference range of noninfected subjects (cut-off) recommended by the manufacturers were considered as positive. The patients of group 3 and controls were defined to have no laboratory evidence of *M. pneumoniae* infection when they showed negative serologic results: neither specific IgG nor IgM response on admission. IgM antibody is known to appear during the first week of illness (18), and all patients of group 3 were transferred from other clinics and had had a history of illness for more than 1 wk on admission.

All patients in our study were studied to find out any co-infecting agent; the studies for respiratory syncytial virus, adenovirus, influenza virus and *Streptococcus pneumoniae* were done. None of groups 1 and 2 had evidences of co-infection. Among 11 patients of group 3, seven patients showed positive results to test for *S. pneumoniae* antigen in urine (Binax, Portland, ME) and no pathogen was identified in the remaining four patients.

Evaluation of serum ET-1, IL-5 and IL-18 levels

Serum ET-1 was determined using the chemiluminescent ELISA (R&D, Minneapolis, MN). IL-5 and IL-18 were determined using the colorimetric sandwich ELISA (R&D, Minneapolis, MN). ELISA sensitivity was 0.16 pg/ml for ET-1, 3 pg/ml for IL-5 and 12.5 pg/ml for IL-18, respectively.

Total serum IgE levels were determined using PRIST (Pharmacia Diagnostics, Sweden).

Statistical analysis

Significant differences were analyzed by ANOVA followed by Duncan post hoc test for multiple

comparisons. Associations were evaluated using Pearson's correlation coefficient. A $p < 0.05$ was considered statistically significant. Data are given as mean $\pm$ SD.

Results

There were no significant difference in age and sex ratio among three patient groups and controls. The clinical characteristics of each patient group and controls were presented in Table 1. The patients with *M. pneumoniae* pneumonia and wheeze needed longer hospitalization and were considered to have a more severe course than the patients without wheeze.

Circulating ET-1 level in each patient group was 16.0 ± 10.4 pg/ml in group 1, 9.7 ± 7.3 pg/ml in group 2, and 5.2 ± 4.4 pg/ml in group 3, which was significantly higher than 0.6 ± 0.2 pg/ml in controls, respectively ($p < 0.01$) (Fig. 1). There was a significant difference between the patients with *M. pneumoniae* pneumonia (group 1 + group 2) and the patients without *M. pneumoniae* infection (group 3) (13.2 ± 9.5 vs 5.2 ± 4.4 , $p < 0.01$) (Table 2). In the presence of *M. pneumoniae* pneumonia, ET-1 in the patients with wheeze (group 1) was significantly higher than in the patients without wheeze (group 2) (16.0 ± 10.4 vs. 9.7 ± 7.3 pg/ml, $p < 0.05$) (Fig. 1).

Serum IL-5 level in each patient group was 40.1 ± 23.6 pg/ml in group 1, 38.8 ± 27.4 pg/ml in group 2, and 26.5 ± 8.1 pg/ml in group 3, which was significantly higher than 11.0 ± 4.7 pg/ml in controls, respectively ($p < 0.01$) (Fig. 2). Serum IL-5 in the patients with *M. pneumoniae* pneumonia (group 1 + group 2) showed a tendency toward higher levels compared with the patients without *M. pneumoniae* infection (group 3), which was not statistically significant (39.5 ± 25.0 vs. 26.5 ± 8.1 pg/ml, $p = 0.09$) (Table 2). There was also a trend toward higher levels in group 1 compared with group 3 (40.1 ± 23.6 vs. 26.5 ± 8.1 pg/ml, $p = 0.07$) (Fig. 2). There was no significant difference

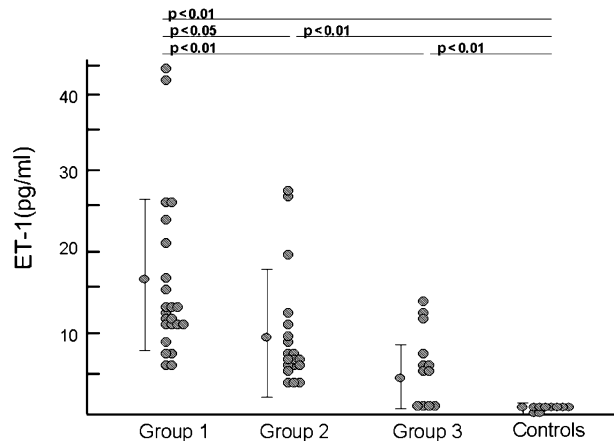


Fig. 1. Serum ET-1 levels in each patient group and controls. ET-1 level in group 1 was significantly higher than in group 2.

between groups 1 and 2 (40.1 ± 23.6 vs. 38.8 ± 27.4 pg/ml, $p = 0.87$) (Fig. 2).

Serum IL-18 in each patient group was 405.9 ± 10.4 pg/ml in group 1, 373.0 ± 166.4 pg/ml in group 2, and 378.0 ± 177.0 pg/ml in group 3, which was significantly higher than 109.4 ± 69.9 pg/ml in controls, respectively ($p < 0.01$) (Fig. 3). No significant between-patient group difference was found (Table 2 and Fig. 3).

The patients in group 1 presented significantly higher levels of total serum IgE compared with groups 2 and 3 (191.1 ± 131.8 vs. 81.0 ± 63.0 IU/ml, 191.1 ± 131.8 vs. 62.2 ± 47.2 IU/ml, $p < 0.05$, respectively) (Table 1). A positive correlation was observed between serum log IgE and ET-1 in group 1 ($r = 0.86$, $p < 0.01$) (Fig. 4), but not in groups 2 and 3.

Discussion

The present study demonstrated significantly increased circulating ET-1 in the patients with *M. pneumoniae* pneumonia. It was also shown that wheezy patients had higher ET-1 levels compared to non-wheezy ones in the course of

Table 1. Clinical characteristics of each patient group and controls

	Group 1 (n = 23)	Group 2 (n = 19)	Group 3 (n = 11)	Controls (n = 10)
Age (yr)	3–12 (4.8)	3–12 (5.2)	4–9 (5.7)	4–10 (6.7)
Sex (M/F)	15/8	11/8	6/5	7/3
Serum IgE (IU/mL)	$195 \pm 131.8^\dagger$	81.0 ± 63.0	62.2 ± 47.2	34.5 ± 12.8
Admission days	$8.9^\ddagger$	6.5	7.2	

† vs. group 2.

‡ vs group 3.

$p < 0.05$.

Table 2. ET-1, IL-5, and IL-18 in the patients with vs without *M. pneumoniae* pneumonia

	ET-1 (pg/ml)	IL-5 (pg/ml)	IL-18 (pg/ml)
Patients with <i>M. pneumoniae</i> pneumonia (groups 1 and 2, n = 42)	13.2 ± 9.5	39.5 ± 25.0	391.2 ± 159.1
Patients without <i>M. pneumoniae</i> infection (group 3, n = 11)	5.2 ± 4.4	26.5 ± 8.1	378.0 ± 177.0
p value	<0.01	0.09	NS

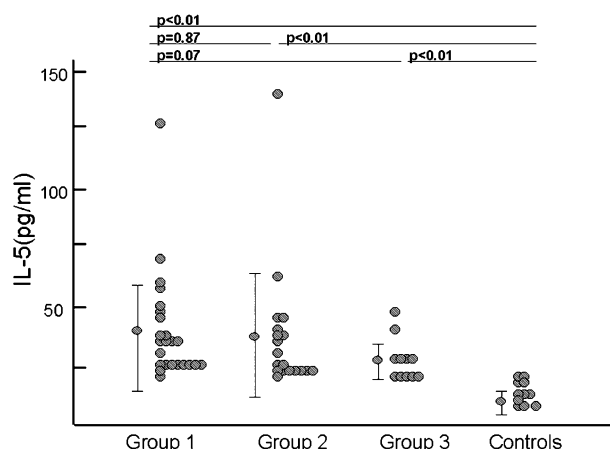


Fig. 2. Serum IL-5 levels in each patient group and controls. There was no significant difference between groups 1 and 2.

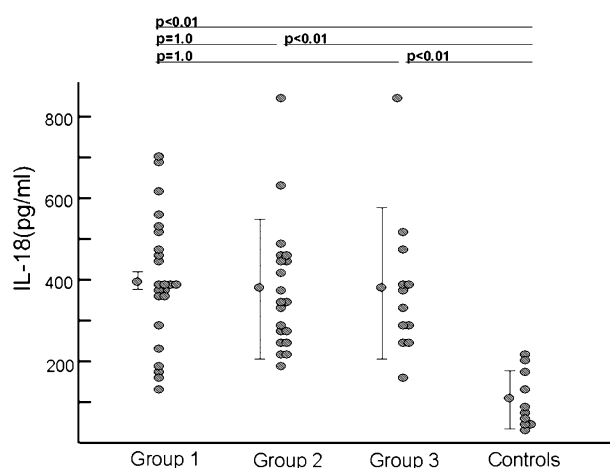


Fig. 3. Serum IL-18 levels in each patient group and controls. There was no significant difference between groups 1 and 2.

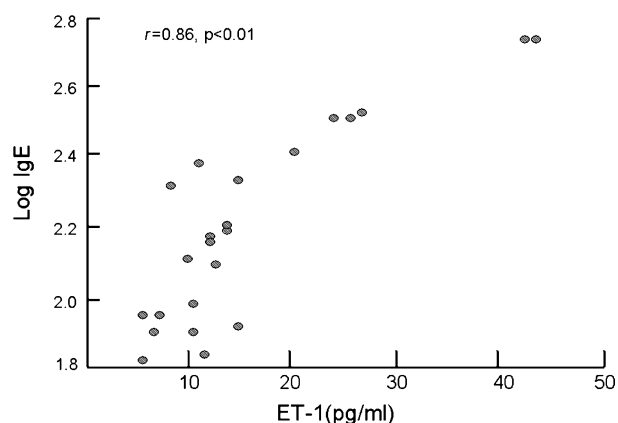


Fig. 4. A positive correlation was observed between serum log IgE and ET-1 in group 1.

acute infection. To our knowledge, this study is the first report concerning ET-1 and *M. pneumoniae* pneumonia in children.

The pathological consequences of mycoplasma infection are not yet clearly understood. Mycoplasma is known to adhere to bronchial epithelial and endothelial cells. An *in vitro* study showed an enhanced ET-1 biosynthesis with mycoplasma infection in the culture of human endothelial cells (11). ET-1 is also a highly potent bronchoconstrictor (10) and can stimulate mucus secretion and edema formation in airways (19, 20). Several *in vitro* and animal studies reported the association between viral respiratory infections and increased ET-1 in the lung and suggested the possible role of ET-1 in the virus-induced airway hyperresponsiveness or exacerbation of asthma (21, 22). Our study showed significantly higher serum ET-1 in the patients with *M. pneumoniae* pneumonia compared with the patients without the evidence of mycoplasma infection, which may suggest a role of ET-1 in the mechanism of airway inflammation during acute stage of *M. pneumoniae* pneumonia.

ET-1 has proinflammatory properties, which contribute to the late-phase allergic response in airways and induces significant eosinophil migration (23, 24). A recent study showed that ET-1 and IL-5 synergistically increase the expression of IL-13 in eosinophils and play prominent roles in allergic inflammation (25). There were other studies about the associations between total serum IgE and polymorphisms in ET-1 gene, which suggest a role of ET-1 in the pathogenesis of atopic disease (26, 27). In reverse, there was a study which showed a significantly enhanced effect of ET-1 on mast cell degranulation when mast cells had been pretreated with IgE (28). This study indicated that a more increased allergic response might be induced by ET-1 in an allergic microenvironment. In our study, both total serum IgE and ET-1 were significantly higher in the patients with wheeze than in the patients without wheeze. There was also a positive correlation between IgE and ET-1 in wheezy patients. The relation between high IgE levels and the occurrence of wheezing in the course of *M. pneumoniae* infection was already reported by a previous study (29). Our results may suggest a role of ET-1 in the pathogenesis of acute wheezing or exacerbation of asthma associated with *M. pneumoniae* pneumonia in the patients with atopic predisposition.

A recent study showed the relation between increased serum IL-5 and wheeze-related symptoms in children with *M. pneumoniae* infection (13). In our study, IL-5 in the patients with *M.*

pneumoniae pneumonia and wheeze also showed a tendency toward higher levels compared with the patients without *M. pneumoniae* infection, but the difference was not statistically significant. A larger study may be needed to determine the role of IL-5 in *M. pneumoniae* pneumonia in relation to the occurrence of wheezing.

IL-18 was found to increase in the serum and pleural fluid of the children with *M. pneumoniae* pneumonia and serum IL-18 correlated well with the severity of disease in the adult patients (15). In our study, serum IL-18 also increased significantly in all patient groups, but no difference was observed between the patients with and without *M. pneumoniae* infection. Our findings support the previous one which showed that IL-18 and Th1 immune response may play an important role in the pathogenesis of *M. pneumoniae* pneumonia (15) and agree with other studies which reported non-specific increase of IL-18 in various lung disease including pneumonia (30, 31).

In conclusion, the present study demonstrated significantly increased serum ET-1 in the children with acute *M. pneumoniae* pneumonia, which was significantly higher in the patients with wheezing. The wheezy patients also had higher IgE levels compared to non-wheezy ones showing a positive correlation between ET-1 and IgE levels. Our results may suggest a role of ET-1 in the occurrence of acute wheezing or exacerbation of asthma associated with *M. pneumoniae* pneumonia.

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