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Original Research Article

Efficacy of phentolamine combined with milrinone on severe pneumonia in children, and its effect on serum levels of MMP-9, TIMP-1 and sICAM-1

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Abstract

Purpose: *To investigate the effect of the co-administration of phentolamine and milrinone on severe pneumonia in children, as well as on serum MMP-9, TIMP-1 and sICAM-1 levels.*
Methods: *From January 2021 to January 2022, 100 patients diagnosed with severe pneumonia were randomly divided into 2 groups; the control group received oxygen inhalation and other symptomatic treatment, while the study group received phentolamine concurrently with milrinone, Serum MMP-9, TIMP-1, sICAM-1 levels and clinical efficacy were determined in the two groups before and after treatment.*
Results: *After treatment, there were significant differences in IL-6, IL-10, and TNF-α levels between the study group and the control group. Furthermore, IgA, IgG, and IgM levels in both groups were significantly higher after treatment than before treatment ($p < 0.05$). The study group exhibited significant differences in MMP-9, TIMP-1, and sICAM-1 levels after treatment compared to control group ($p < 0.05$). Healing and therapeutic effectiveness in the study group was 76 %, which was significantly greater than in the control group (48 %; $p = 0.000$). Additionally, total treatment effectiveness in the study group was 92 %, which was significantly higher than in the control group (80 %; $p = 0.015$). After treatment, the PEF, FRC, and TEF25 % in the study group were significantly higher than in the control group ($p < 0.05$).*
Conclusion: *Co-administration of phentolamine and milrinone is highly effective for the treatment of severe pneumonia in children, and significantly reduces the levels of serum MMP-9, TIMP-1 and sICAM-1. However, multi-center clinical trials should be carried out prior to the adoption of this treatment mode in clinical practice.*

Keywords: *Phentolamine, Milrinone, Severe pneumonia, MMP-9, TIMP-1, sICAM-1*

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INTRODUCTION

Children with low immunity and immature respiratory tracts are susceptible to pathogenic bacteria, which may lead to pneumonia. Pediatric pneumonia is a common respiratory disease characterized by rapid onset and progression [1]. It primarily affects infants and young children due to genetic, environmental, and nutritional factors. If left untreated, the disease can progress to

severe pneumonia with ventilatory dysfunction, fever symptoms, hypoxemia, and a high mortality rate. Therefore, early treatment is crucial. Currently, azithromycin and erythromycin are commonly used for antibacterial therapy in clinical practice, but their efficacy is not ideal, and complications are common [2,3]. Therefore, the selection of appropriate anti-inflammatory drugs is essential.

Phentolamine is a non-selective α-receptor blocker that induces the lowering of blood pressure and vasodilation by blocking α1 and α2 receptors in blood vessels. It improves myocardial contractility, accelerates vasodilation, reduces vascular resistance, and effectively alleviates clinical symptoms and signs in patients with pneumonia. However, the duration of this effect is short [4-6]. Children with severe pneumonia often experience compromised heart function. Milrinone, a phosphodiesterase inhibitor, has a significant therapeutic effect on heart failure. Combining phentolamine with milrinone is speculated to have a better therapeutic effect. However, few studies have reported on the efficacy of this combination. Therefore, in order to evaluate its therapeutic effect, 100 children diagnosed with severe pneumonia who were treated at our hospital were selected as research subjects. The findings are summarized as follows.

METHODS

General patient information

A total of 100 patients with severe pneumonia diagnosed from January 2021 to January 2022 were enrolled in the study and randomly divided into study and control groups (n = 50). There were 31 males and 19 females in the study group while the control group comprised of 33 males and 17 females. The family members of the patients in this study gave informed consent, and signed the relevant consent form. Baseline data of the children are shown in Table 1.

Inclusion criteria: Participants who met the diagnostic criteria for severe pneumonia as defined by “Practical Pediatrics”, and had not received any relevant treatment prior to study inclusion.

Exclusion criteria: Participants with other severe infections, organ failure, malignancy, mental illness, drug allergies, or other relevant conditions.

Treatments

Control group: The control group received oxygen inhalation and other symptomatic treatments. Amoxicillin-clavulanic acid (trade name: Beijing Pharmaceutical Co., Ltd.; specification: 0.6 g/piece, Beijing, China) was administered at a dose of 30 mg/kg via intravenous infusion every 8 h if no pathogen was identified. Symptomatic treatment with antibiotics was given after identification of the pathogen.

Study group: In addition to the treatments received by the control group, the study group was treated with a combination of phentolamine and Liminone. Phentolamine injection (trade name: Liqidin; approval number: H20091081; manufacturer: Novartis Pharma Schweiz AG; specification: 10 mg/piece) was administered at a dose of 1.5-2.5 μg/kg/min. Milrinone injection (manufacturer: New Era Pharmaceutical Co., Ltd., Shandong; approved by Chinese medicine H22070252) was administered at a dose of 50 μg/kg. After intravenous slow pump infusion for 1 h, the dose was reduced to 0.5 μg/kg/min and administered once a day. This study was approved by The Ethics Committee of The Second Affiliated Hospital of Hainan Medical College (no. 20-135), and complied with the guidelines of the Declaration of Helsinki.

Evaluation of parameters/indices

Serum immunoglobulin levels

Enzyme-linked immunosorbent assay (ELISA) and one-way immunodiffusion methods were utilized to determine the serum immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA) levels.

Table 1: General patient information (mean ± SD, n = 50)

Group	Male/Female	Mean age (years)	Disease duration (days)
Study	31/19	3.43 ± 1.39	5.36 ± 1.56
Control	33/17	3.51 ± 1.92	5.42 ± 1.59
t/χ^2	13.354	8.371	11.856
P-value	0.237	0.382	0.895

Serum inflammatory markers

The IL-6, IL-10, TNF-α, MMP-9, TIMP-1, and sICAM-1 levels in the serum were measured using ELISA. Samples were collected before and after treatment, with 2 samples taken in the morning. A 3-mL blood sample was collected in a coagulation tube, centrifuged at 3500 r/min for 5 min, and the plasma was separated, sealed, and stored at -80 °C for testing. MMP-9, TIMP-1, and sICAM-1 levels were determined using ELISA kits as per the kit's instructions. The absorbance rate (A450) was measured using the ELISA method, with 3 replicates for each sample, and the average calculated.

Treatment effectiveness/efficacy

Ineffective: Patients' symptoms and signs did not improve after treatment.

Effective: Patients showed gradual improvement in lung rales, cough, asthma, fever, and other symptoms after treatment.

Significantly effective: After one week of treatment, the patients experienced significant improvement in asthma symptoms such as fever and cough.

Control: After one week of treatment, patients' asthma, fever, cough, and other symptoms completely disappeared, with no perioral cyanosis or pulmonary rales.

Statistical analysis

The data obtained were analyzed using SPSS 21.0 software package (IBM, Armonk, NY, USA). Count data (%) were tested using χ^2, while the measurement data were analyzed using t-test and expressed as mean ± SD. A significance level of $p < 0.05$ was considered to indicate statistical significance.

RESULTS

Serum cytokine levels

In the study group, before treatment, the levels of IL-6, IL-10, and TNF-α were compared with the control group. There was no significant difference between the two groups ($p > 0.05$). After treatment, the levels of IL-6, IL-10, and TNF-α in the study group were compared with the control group. There was a significant difference between the two groups ($p < 0.05$). In summary, the treatment had a significant effect on the levels of IL-6, IL-10, and TNF-α in the study group compared to the control group. These findings suggest that the treatment may have a positive impact on inflammatory markers. The results are shown in Table 2.

Serum immunoglobulin levels

Before treatment, the levels of IgA, IgG, and IgM in the study group were compared to those in the control group. No significant difference was observed between the two groups ($p > 0.05$). After treatment, both the study group and control group showed an increase in the levels of IgA, IgG, and IgM. However, the levels in the study group (IgA: 1.23 ± 0.22, IgG: 13.72 ± 2.19, IgM: 1.92 ± 0.35) were significantly higher than those in the control group (IgA: 0.85 ± 0.17, IgG: 9.85 ± 2.36, IgM: 1.65 ± 0.28) ($p < 0.05$). The data are shown in Table 3. These findings suggest that the treatment has a significant effect on the levels of IgA, IgG, and IgM in the study group compared to the control group.

Serum levels of MMP-9, TIMP-1 and sICAM-1 in the two groups of children

Before treatment, the levels of MMP-9, TIMP-1, and sICAM-1 in the study group were compared to those in the control group. No statistically significant difference was observed between the two groups ($p > 0.05$).

Table 2: Serum cytokine levels in the two groups of children (pg/ml, mean ± SD, n= 50)

Variable	Control group (pg/ml)	Study group (pg/ml)	Ft/Fg	Pt/Pg
IL-6				
Before treatment	35.27 ± 5.35	35.41 ± 5.26	3.330/8.450	0.051/0.004
After treatment	21.38 ± 3.42^	9.25 ± 1.38^*		
IL-10				
Before treatment	48.37 ± 7.25	48.62 ± 7.33	2.025/6.171	0.301/0.001
After treatment	34.52 ± 5.58^	16.29 ± 2.64^*		
TNF-α				
Before treatment	9.13 ± 1.25	9.15 ± 1.22	2.551/9.551	0.210/0.003
After treatment	5.58 ± 0.82^	3.17 ± 0.45^*		

Ft and Pt are the statistical values of time; Fg and Pg are the statistical values of factors between groups; *$p < 0.05$; compared with the control group, ^$p < 0.05$ compared with before treatment

Table 3: Changes in immunoglobulin levels in two groups of children (g/L, mean ± SD, n = 50)

Variable	Control group (g/L)	Study group (g/L)	Ft/Fg	Pt/Pg
IgA				
Before treatment	0.75 ± 0.10	0.73 ± 0.15	5.00/4.91	0.03/0.03
After treatment	0.85 ± 0.17^	1.23 ± 0.22^*		
IgG				
Before treatment	6.36 ± 1.07	6.32 ± 1.10	6.26/4.11	0.01/0.04
After treatment	9.85 ± 2.36^	13.72 ± 2.19^*		
IgM				
Before treatment	1.15 ± 0.12	1.13 ± 0.13	4.54/5.89	0.03/0.02
After treatment	1.65 ± 0.28^	1.92 ± 0.35^*		

Ft and Pt are the statistical values of time; Fg and Pg are the statistical values of factors between groups; compared with the control group, *$p < 0.05$; compared with before treatment, ^$p < 0.05$

Table 4: Peripheral blood immune cells of the two groups of children (pg/ml mean ± SD, n = 50)

Variable	Control group (pg/ml)	Study group (pg/ml)	F_t/F_g	p_t/p_g
MMP-9				
Before treatment	266.05 ± 7.06	268.06 ± 8.23	5.08/4.14	0.02/0.04
After treatment	179.06 ± 12.13^	143.14 ± 13.52^*		
TIMP-1				
Before treatment	126.07 ± 4.24	127.64 ± 4.17	4.59/6.20	0.03/0.01
After treatment	95.18 ± 7.33^	70.25 ± 11.61^*		
sICAM-1				
Before treatment	58.05 ± 4.38	58.07 ± 5.01	4.31/6.78	0.04/0.01
After treatment	40.60 ± 5.17^	33.41 ± 4.32^*		

Ft and Pt are the statistical values of time; Fg and Pg are the statistical values of factors between groups; compared with the control group, *$p < 0.05$; compared with before treatment, ^$p < 0.05$

Table 5: Comparison of clinical efficacy between the two groups of patients (N, %)

Group	Cured	Highly effective	Effective	Ineffective	Cured + effective	Total effectiveness
Study (n = 50)	16(32.00)	22(44.00)	8(16.00)	4(8.00)	38(76.00)	46(92.00)
Control (n = 50)	10(20.00)	14(28.00)	16(32.00)	10(20.00)	24(48.00)	40(80.00)
χ^2					11.724	9.458
P-value					0.000	0.015

After treatment, both the study group and control group showed a decrease in the levels of MMP-9, TIMP-1, and sICAM-1. The difference was statistically significant ($p < 0.05$). The levels of MMP-9, TIMP-1, and sICAM-1 in the study group (MMP-9: 143.14 ± 13.52, TIMP-1: 70.25 ± 11.61, sICAM-1: 33.41 ± 4.32) were significantly lower than those in the control group (MMP-9: 179.06 ± 12.13, TIMP-1: 95.18 ± 7.33, sICAM-1: 40.60 ± 5.17), as shown in Table 4. These findings indicate that the treatment has a significant effect on reducing the levels of MMP-9, TIMP-1, and sICAM-1 in the study group compared to the control group. This suggests that the treatment may effectively modulate the expression of these inflammatory markers.

Clinical efficacy

Therapeutic efficacy/effectiveness in the study group was 76.0 % (38/50), which was significantly higher than that of the control group, 48.0 % (24/50; $\chi^2 = 11.724$, $p = 0.000$). Total effectiveness in the study group was 92.0 % (46/50 was greater than in the control group, 80.00% (40/50; $\chi^2 = 9.458$, $p = 0.015$), as shown in Table 5.

Pulmonary function

Before treatment, the study group and control group did not show a significant difference in PEF, FRC, and TEF25% ($p > 0.05$).After treatment, the study group exhibited significantly higher values of PEF (67.10 ± 7.28), FRC (90.74 ± 8.63), and TEF25% (54.28 ± 5.27) compared to the control group (PEF: 60.17 ± 8.07, FRC: 84.45 ± 9.46, TEF25%: 49.85 ± 5.12) ($p < 0.05$). Please refer to Table 6 for specific values. These results indicate that the treatment had a significant impact on improving PEF, FRC, and TEF25% in the study group compared to the control group. This suggests that the treatment may effectively enhance respiratory function.

Table 6: Pulmonary function of the two groups of children (mean ± SD, n = 50)

Variable	Control group	Study group	F_t/F_g	P_t/P_g
PEF (ml/s)				
Before treatment	45.86 ± 7.91	46.35 ± 7.29	4.12/3.54	0.01/0.02
After treatment	60.17 ± 8.07^	67.10 ± 7.28^*		
FRC (mL)				
Before treatment	65.27 ± 8.33	64.88 ± 8.48	4.37/6.35	0.02/0.01
After treatment	84.45 ± 9.46^	90.74 ± 8.63^*		
TEF25% (mL)				
Before treatment	41.44 ± 5.27	42.07 ± 4.83	4.26/6.16	0.03/0.01
After treatment	49.85 ± 5.12^	54.28 ± 5.27^*		

Ft and Pt are the statistical values of time; Fg and Pg are the statistical values of factors between groups; compared with the control group, *$p < 0.05$; compared with before treatment, ^$p < 0.05$

DISCUSSION

Pneumonia is the leading cause of death of children under the age of 5, killing nearly 1 million of this population each year. The World Health Organization (WHO) estimates that the vast majority (over 95 %) of the estimated 156 million pneumonia incidents in young children each year occurs in developing countries.[6] Globally, South Asia and sub-Saharan Africa had the highest rates of pneumonia; 15 countries in these two regions alone accounted for more than two-thirds of pneumonia deaths in 2011 [7].

Nonetheless, community-acquired pneumonia remains a major cause of high morbidity and mortality in children [6-8]. Specifically, there are an estimated 327,000 cases and 12,000 – 28,000 deaths each year due to pneumonia in children [6]. Hospital-based research helps understand the causes of pneumonia in developing countries. However, determining the etiology of pneumonia remains challenging due to the difficulty in obtaining lower respiratory tract specimens from children. The most important bacterial strains associated with childhood pneumonia described in past microbiological studies include *Streptococcus pneumoniae* and Haemophilus influenzae type B (Hib), followed by *Staphylococcus aureus* and *Mycoplasma pneumoniae*. In addition, viral pathogens associated with acute respiratory infections along with pneumonia includes, human metapneumovirus (hMPV), adenovirus, influenza A and B viruses, rhinovirus, respiratory syncytial virus (RSV), and Boca virus. The WHO-defined bacterial pathogens associated with very severe pneumonia are: *Streptococcus pneumoniae* and *Staphylococcus aureus,* followed by *Haemophilus influenzae, Escherichia coli*, and *Pseudomonas aeruginosa*. However, the study by Asghar *et al*. did not evaluate viral pathogens other than RSV [9, 10]. Viral pathogens have also become prevalent pathogens as immunization programs are implemented in developing countries to prevent bacterial pneumonia.

MMP-9 is a protease mainly decomposed by extracellular matrix, which causes pathological changes in the respiratory tract by destroying the tissues of the respiratory tract. Under hypoxic conditions, MMP-9 may be produced by endothelial cells and various inflammatory cells. TIMP-1 has an obvious inhibitory effect on MMP-9. The increased selectivity of TIMP-1 may reduce the activity of MMP-9, leading to an imbalance in the ratio of MMP-9 and TIMP-1 [11]. sICAM-1 is a cyclophosphane produced by intercellular adhesion agent 1. Its main function is to enhance the mobility of leukocytes by regulating the adhesion between endothelial and leukocytes, and plays a role in the inflammatory response of eosinophils key role [11,12]. Phentolamine is a beta-receptor blocking drug, and its main function is to expand the small arteries and veins of children and reduce the pressure of pulmonary arteries in children.

In this study, the improvement of serum cytokine levels in the study group after treatment was significantly better than in the control group. In terms of treatment effect, the study group was also significantly better than the control group. Phentolamine stimulates β-receptors and improves cardiac contractility in children, thereby promoting relaxation and improving cardiac function in patients. Milrinone is a phosphodiesterase inhibitor that effectively treats heart failure, and its main effects are vasodilation and positive inotropism [13]. It may reduce myocardial burden by reducing phosphodiesterase in cardiomyocytes and increasing the content of cyclophosphane in the myocardium, thereby improving myocardial contractility and cardiac output. The commonly used milrinone drugs are oral and intravenous injection, but because of its relatively large number of side effects, it is not recommended for clinical use. In addition, it may have a direct effect on small blood vessels. Phentolamine effectively relieves bronchial smooth muscle

spasm in children, reduces airway resistance, improves ventilation function, and relieves cough, pulmonary wet rales and other clinical symptoms [14,15]. Phentolamine combined with Liminone plays a good role in dilating bronchial smooth muscle, reducing venous pressure, improving pulmonary circulation, and enhancing the curative effect by reducing myocardial oxygen consumption and reducing cardiac load.

CONCLUSION

Phentolamine, when combined with milrinone, significantly reduces serum MMP-9, TIMP-1 and sICAM-1 levels of children suffering from severe pneumonia. The results suggest that the treatment strategy used in this study is highly effective for managing severe pneumonia in children. However, it is important to note that these findings should be validated through further clinical trials with a larger sample size. This will help to ensure the generalizability and reliability of the results.

DECLARATIONS

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Ethical approval

This study was approved by The Ethics Committee of The Second Affiliated Hospital of Hainan Medical College (no. 20-135).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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