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Expressions and Significance of the Inflammatory Factors in Serum of Children with *Mycoplasma Pneumoniae* Pneumonia*

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ABSTRACT Objective: To detect the levels of IL-8 and IL-12 in cytokine and to observe the the changes of hs-CRP Ig and complement (C) in serum of children with the MPP. **Methods:** 50 children with MPP were selected and divided into the severe group and the mild group. Another 42 healthy children were chosen to be the control group. Then the levels of IL-8 and IL-12 in the serum of children in the control group and the children with MPP in the acute and recovery phase by means of ELISA. The changes of hs-CRP, lg and C in serum were detected by Plasma protein analyzer and rate nephelometry. **Results:** In the acute and recovery phase of MPP, the expression of IL-12 was apparently lower than that of the control group (P<0.05), while the expression of IL-8 was significantly higher than that of the control group (P<0.01). The expression of IL-12 in the serum of the children in the severe group was markedly lower than that of the mild group, while the expression of IL-8 was higher than that of the mild group (P<0.01). In the acute phase, the changes of IgM and IgG in the serum of children with MPP were up-regulated and the changes of IgA was down-regulated(P<0.05). In the severe phase, the levels of hs-CRP, C3 and C4 in the serum of children with MPP increased when comparing with the control group(P<0.01). IgM and IgG in the serum of the acute group significantly increased, and the IgA decreased when comparing with the mild group; hs-CRP, C3 and C4 in the serum of the severe group significantly increased when comparing with the mild group (P<0.01). **Conclusion:** It is indicated that the detection of inflammatory cytokines in the serum of children with MMP could be helpful to the determination and prognosis of the disease.

Key words: Pneumonia; Mycoplasma/blood; Serum Inflammatory Factors; Clinic Significance

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Introduction

Mycoplasma pneumoniae (MP) is a kind of pathogens between bacteria and viruses, and is one common pathogen in respiratory tract infection in children. It is also one of the most important pathogens that cause paediatric lower respiratory tract infection. The morbidity rate in our country is 19.6%-21.9%, and in peak year it will reach as high as 33.1%[1]. Not only MP induce the inflammation of respiratory system, it can also lead to the extra-pulmonary complications [2], and be closely to the incident of asthma [3]. Therefore, it becomes a hot-spot of research to explore the pathagenesis of mycoplasma pneumonia so as to explore the new effective measures for the clinical treatment. This experiment studies the level of the inflammatory cytokines interleukin in the serum (IL-8 and IL-12), immunoglobulin (IgA, IgG and IgM), complement (C3 and C4) and high-sensitivity C-reactive protein (hs-CRP) by ELISA and plasma protein analyzer rate nephelometry. The aim is to probe the changes and significance of the cell factor cytokine, lg, and acute phase reaction protein in children with MPP in acute and recovery phase.

1 Materials and Methods

1.1 Clinical data

50 cases were chosen from hospitalized children with MPP in this hospital, 27 children are male and 23 are female. The average age is 1-14 (5.3± 2.9), which complies with the diagnostic standard of *Mycoplasmal Pneumonia* (MPP) <practical paidonosology> [4]. They are divided into severe group and mild group with 28 and 22 patients, respectively. Altogether 42 healthy patients were selected as the control group with 24 male and 18 female. The average age is 2～13 (6.1± 3.2), these children are all examined as healthy by the hospital, and in the recent 3 months and there was no previous history of acute or chronic disease was confirmed.

1.2 Sample collection

To collect 3mL veinous blood on an empty stomach in the morning from the acute group, recovery group and control group respectively, 4000 r/min centrifugally separated for 5 min and then use the abstracted serum, which should be reserved in the refrigerator at minus 30 degrees Celsius for examination.

1.3 Detection methods

① The examination of MP pathogeny applies test reagent made by Japan's Fuji antibody against *mycoplasma pneumoniae* which is purchased from Guangzhou Zhi Jia Technology CO., LT. It utilizes low titer gelatin particle to conduct indirect haemagglutiantion test. Results Decision: After operation, put it in the room temperature of 15℃ ～30℃ for 3h,and then observe the results, with titers (1:1280) as terminal point. Feminine≤ 1:40; Masculine≥ 1:80. ② Component Detection of hs-CRP, serum Ig and C:

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to test by Plasma protein analyzer and rate nephelometry on the Behring nephelometer systems (BNⅡ), and the used reagent is Behring original reagent. ③ Detection of IL-8 and IL-12: both apply the approach of ELISA, and the reagent kit is purchased from Shenzhen Jinmei Biological Technology Co., Ltd. The operation is conducted strictly in accordance with the instruction of the reagent kit.

1.4 Statistical Analysis

SPSS13.0 software packet was used to complete the statistical analysis, and the data is expressed as. The set of data should apply T test of paired samples.

2 Results

2.1 Expressions of IL-8 and IL-12 in different groups

Comparing with the control group, the content of IL-12 in serum of the children with MPP in the acute phase was significantly lower ($P<0.01$), while the content of serum IL-12 in the recovery phase increased, but still ($P<0.05$). In comparison with the content of serum IL-8 of the children of control group, that of the children with MPP in the acute phase was significantly higher ($P<0.01$), while compared with the content of serum IL-8 of the children of control group, the children with MPP in the recovery phase decreased apparently ($P>0.05$). There were no significant differences between serum IL-8 and IL-12 in the acute phase and recovery phase, respectively($P<0.01$, $P<0.05$). (Table 1).

2.2 IL-8 and IL-12 in serum between the severe group and the mild group

Among the MPP children, the expressions of IL-12 in the children of the severe group is lower than that of the mild group ($P<0.01$), while the serum IL-8 of the severe group is higher than that of the mild group ($P<0.01$), (Table 2).

Table 1 Detection Results of Serum IL-8 and IL-12 in children with MPP ($\bar{x}\pm s$, pg / mL)

Group	n	IL-8	IL-12
Acute Phase	50	110.60± 20.16△▲	102.3± 53.16△※
Recovery Phase	50	28.06± 27.83	113.61± 17.63*
Control Group	42	24.84± 18.35	132.12± 48.64

Note: △Compared with the control group, $P<0.01$, *compared with the control group, $P<0.05$.
▲ compared with the recovery phase, $P<0.01$, ※ compared with the recovery phase, $P<0.05$.

Table 2 Detection of Serum IL-8 and IL-12 in the Severe Group and the Mild Group of the Children with MPP

Groups	n	IL-8	IL-12
The Mild Group	22	96.51± 28.65	115.4± 28.65
The Severe Group	28	108.47± 37.34*	97.54± 31.04△

Note: △Compared with the mild group, $P<0.01$. *compared with the mild group, $P<0.05$.

2.3 Immunoglobulin of children with MPP

Compared with the serum immunoglobulin IgM and IgG of the control group, those of the children with MPP in the acute phase significantly increases ($P<0.01$), while IgA of the children with MPP in the acute phase significantly decreases ($P<0.05$); In comparison with the control group in the recovery phase, IgG is still high, while there are no obvious changes that occur to the serum IgA and IgM ($P>0.05$). In comparison with the control group, hs-CRP and C3 in the children with MPP in the acute phase increase significantly ($P<0.01$), while C4 increase significantly ($P<0.05$). The hs-CRP in the recovery phase increase significantly in comparison with that in the control group ($P<0.05$); In comparison with the recovery phase, hs-CRP and C3 in the children with MPP in the acute phase is apparently higher($P<0.01$). (Table 3).

Table 3 Results of Complete Detection of Serum Immunoglobulin of Children with MPP ($\bar{x}\pm s$,g / L)

Groups	n	hs-CRP	IgA	IgM	IgG	C3	C4
The Control Group	42	1.54± 1.27	1.28± 0.77	0.86± 0.42	4.15± 3.27	1.07± 0.25	0.31± 0.089
The Acute Phase	50	32.0± 26.26△▲	1.05± 0.46*	1.64± 0.46△	9.75± 2.93△	1.78± 0.17△▲	0.34± 0.15△
The Recovery Phase	50	3.31± 1.94*	1.10± 0.48	0.91± 0.50	7.64± 1.91△	1.16± 0.38	0.33± 0.142

Note: Compared with the control group, △$P<0.01$, *$P<0.05$
▲Compared with the recovery phase: $P<0.01$.

2.4 Immunoglobulin in the severe group and the mild Group

The serum immunoglobulin IgG and IgM in the severe group of the children with MPP significantly increase compared with that in the mild group ($P<0.01$) while IgA significantly decreases ($P<0.05$). Serum CRP, C3 and C4 in the severe group of the children with MPP significantly increase compared with that in the mild group. (Table 4).

Table 4 Results of the Complete Detection of Serum Immunoglobulin in the Severe Group and the Mild Group of the Children with MPP ($\bar{x}\pm s$,g / L)

Groups	n	hs-CRP	IgA	IgM	IgG	C3	C4
The Mild Group	22	19.6± 9.23	0.78± 0.50	1.27± 0.52	7.91± 2.84	1.54± 0.16	0.29± 0.12
The Severe Group	28	51.48± 25.0△	0.64± 0.44*	1.76± 0.58△	9.84± 1.61△	1.91± 0.21△	0.49± 0.14△

Note: Compared with the mild group, △$P<0.01$; *$P<0.05$.

3 Discussion

As many recent researches show [5,13-15], MP infection can induce the body to produce various cytokines, including IL-2, IL-4, IL-6, IL-8, IL-12, Tumor Necrosis Factor-α (TNF-α)and so on. These cytokines can influence each other, interacting and interre-

lating on each other. They affect a variety of immune globulin, complement and the formation of proteins in acute phase, together with which a complex network system is formed. In this research, it is found that there was abnormal expression in a variety of inflammatory cytokines in serum like IL-8, IL-12, IgA, IgG, IgM and compliments C3 and C4 in children with MPP which indicated that various inflammatory mediators get involved, and there appeared disorder of the body's immunological function. IL-8, mainly the strongest chemotactic and activated factor produced by monocytes, caused neutrophils and T lymphocytes and other inflammatory cells to infiltrate accumulate and release active products in the affected tissue[6]. Thus leading to inflammatory reaction of a body part, and acquire the ability of sterilization and cell injury. This group of experiments shows that in the acute phase IL-8 levels of the severe group of children with MPP significantly increase, and this is possibly that the heterologous antigen, MP goes into circulation of blood, and then stimulate T and B lymphocytes, monocyte-macrophages, neutrophils, vascular endothelial cells and the secretion of IL-8 in blood. At the same time, endotoxin produced by MP, IL-1, TNF-αstimulation can also stimulate in different degrees the secretion of IL-8[7]. The increase of IL-8 can further cause the inflammatory reaction through neutrophil chemotaxis and degranulation, resulting in the occurrence and development of the disease. IL-12 can induce the synthesis of γ-interferon (IFN-γ) and also induce and maintain Th1 cell-mediated immune response, which plays an important role in the early stage of inflammation[8]. This group of experiments indicates that the level of serum IL-12 in children with MPP decrease significantly, and is significantly lower than that in control group. The decrease in the level of IL-12 in children with MPP is not conducive for the transformation of Th0 cell to Th1 cell, which may cause the imbalance of Th1/Th2 cytokine. This also indicates that in the acute phase, the low level of IL-2 in the severe group of children with MPP is possibly related to the disorder of their immunological function and immunological deficiency. Therefore, the detection of cytokines has high clinical value on the determination condition and prognosis. After MP is infected, a series of immune responses will occur in the body's immune system against the intrusion of MP, and this process not only includes cellular immunity of T cells mediated by cytokine, but also includes humoral immune of B cells mediated by Ig. However, the humoral immune that Ig gets involved in plays a secondary role in immunologic mechanism of pathogenesis whereas cytokine or cellular immunity plays a major role [9]. This group of experiment also finds that the content of serum IgG, IgA, IgM, C3, C4 in children with MPP in the acute phase were significantly higher that in the control group, which also indicates that humoral immune did participate while MP was infected. Many scholars believe that there exists some common antigen among MP antigens and human heart, lung, liver, brain, kidney and muscle and other tissues. The common antigen can stimulate B cells to produce IgM and IgG antibodies while MP is infected, and cause changes of a host antigen in the structure to produce its own antibodies under the influence of the complement C3 and C4. These antigen-antibody form immune complex, and then further activate complement and immune cells to play strong immunogenicity effect[10-12]. This not only leads to lung damage, but also causes children with severe illness to suffer from extra-pulmonary of multiple systems. The content of serum complement component C3, C4 in this group of MPP in acute phase was significantly higher than that in the control group. Serum complement was activated when suggesting MPP, and in turn a chain reaction occurs, and the content of serum complement C3, C4 increased which indicates that complement is activated through the traditional way. In this group of experiments, IgG in both the acute and recovery phase is higher than that in the control group, because the IgG disappeared late in Ig. This can neutralize free exotoxin and viruses, conditioning the phagocytic mechanism,which plays an important role in immunity protection. However, IgA, as the membrane surface antibody, has functions of anti-bacterial, anti-toxins, anti-viral, can also play a positive role in mycoplasma and certain fungi. It is found that the low expression of IgA is prone to cause respiratory diseases, and in this group of experiments the level of serum IgA of the children with MPP in the acute phase was found to decrease significantly in comparison with that in the control group, while the content of serum IgA in the severe group is significantly lower than that in the mild group. This indicates that these children have lower resistance, and there are possibilities of disorder of their immunological function and immunological deficiency [14]. However, after infection IgM appeared earlier Ig, and was Ig in the acute phase, but it disappeared very early [15]. From this group of experiments, it can be seen that there are no distinctive differences between its level in the recovery phase and that in the control group. The author observes that the severity of children with MPP relates to the content of serum IgG, IgA, IgM and complement component, suggesting while MP is infected, serum Ig and the changes of complement component have clinical value on determining the severity and prognosis of the disease, and playing an important role in MPP pathogenesis. Besides, from the result of this group of experiment, one or more items of immunoglobulin in children infected by MP is higher than normal, indicating both the hyperpituitarism of B lymphocyte function in children infected by MP and the increase of synthesis of Ig[16-18]. Among the children infected by MP, disorder of immunological function is common. Despite the hyperpituitarism of immunological function among the children infected by MP in the acute phase, the cellular immune function of the majority of children infected by MP are low[19,20]. In this experiment, the level of hs-CRP in the acute phase is at a high level, but in the recovery phase, it significantly decreased, and tended to be normal. The level of the severe group was significantly higher than that in the mild group, suggesting that the level of hs-CRP relate closely to the extent of tissue damage. Clinically severity and prognosis can be promptly determined by detecting changes in hs-CRP and serum Ig and complement levels, and this is helpful to guide the clinical treatment of children infected by MP.

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支原体肺炎患儿血清中相关炎性因子的表达变化情况及其临床意义探讨*

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摘要 目的:探讨支原体肺炎(*Mycoplasma Pneumoniae* Pneumonia, MPP)患儿血清中细胞因子 IL-8,IL-12 的表达水平及 hs-CRP、IgG 和血清补体(C)的变化及其临床意义。**方法:**收集 MPP 患儿 50 例,分为重症组、轻症组。健康儿童 42 例作为对照组;用 ELISA 法测定 MPP 患儿急性期、恢复期及对照组儿童血清 IL-8、IL-12 的水平,用血浆蛋白分析仪速率散射比浊法测定 hs-CRP,Ig 和 C 含量。**结果:**在急性期和恢复期 MPP 患儿血清 IL-12 含量明显低于正常对照组(P<0.05);而血清 IL-8 含量在急性期明显高于正常对照组(P<0.01)。重症组患儿血清中 IL-12 明显低于轻症组,而血清中 IL-8 较轻症组高(P 均 <0.01)。急性期 MPP 患儿血清 IgM,IgG 与对照组相比明显升高(P 均 < 0.01);而 IgA 明显降低(P<0.05)。急性期 MPP 患儿 hs-CRP、C3、C4 与对照组比较显著升高(分别为 P<0.01、P<0.01、P<0.05)。重症组患儿血清中 IgM,IgG 与轻症组相比明显升高(P 均 <0.01);IgA 与轻症组相比明显降低(P<0.05);重症组患儿血清中 hs-CRP、C3、C4 与轻症组相比明显升高(P 均 <0.01)。**结论:**检测相关血清炎性细胞因子对判定 MPP 患儿的病情和预后有较高的临床应用价值。

关键词:肺炎;支原体 / 血液;血清炎性因子;临床意义
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