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Effects of hemodialysis and hemoperfusion on inflammatory factors and nuclear transcription factors in peripheral blood cell of multiple organ dysfunction syndrome

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Abstract. – OBJECTIVE: To investigate the effects of hemodialysis (HD) and hemoperfusion (HP) on inflammatory factors and nuclear transcription factors in peripheral blood cell of multiple organ dysfunction syndrome (MODS) patients.

PATIENTS AND METHODS: 92 cases of MODS patients undergoing maintained hemodialysis in our hospital were randomly divided into the control group and observation group. The control group was treated with conventional hemodialysis (HD), the observation group was treated with hemoperfusion combined therapy (HD+HP) based on the control group. The levels of serum creatinine (SCR), serum total cholesterol (TC), blood urea nitrogen (BUN) and serum albumin (Alb) were compared and analyzed between two groups before and after treatment. The levels of NK-κB p65 in the white cell of peripheral blood were compared between two groups before and after treatment by Western blot. The levels of inflammatory factors TNF-α and IL-6 in peripheral blood were detected between two groups before and after treatment using ELISA method.

RESULTS: Compared with the control group, the levels of Scr, BUN and TC were significantly decreased, while the level of Alb was significantly increased ($p < 0.05$). The level of cytoplasm NK-κB p65 protein in peripheral blood was significantly increased in the observation group, while the level of nuclear NK-κB p65 in peripheral blood was significantly decreased ($p < 0.05$).

CONCLUSIONS: Hemodialysis combined with hemoperfusion in treating MODS patients could significantly improve the biochemical indicators, effectively remove the inflammatory mediums, and significantly inhibit the activation of NK-κB.

Key words:
Hemodialysis, Hemoperfusion, Multiple organ dysfunction syndrome, Inflammatory factors, Nuclear transcription factors.

Introduction

Multiple organ dysfunction syndrome (MODS) is a clinical syndrome characterized by various causes, complex pathogenesis and high mortality. MODS refers to that the body occurs to two or above organ dysfunction at the same time or sequentially after severe diseases, trauma, surgery, infection, shock and other serious damages, that is a clinical syndrome which the homeostasis cannot be maintained even organ failure caused by acute injury[1,2]. According to the literature, the mortality rate of MODS involving only two organs was 50%, the mortality rate of MODS involving kidneys and lungs was as high as 80%, the mortality rate of MODS in involving more than four organs was 100%. With the deep understanding of the nature of MODS, people pay more attention to its immune pathogenesis. Systemic uncontrolled inflammatory response, immune dysfunction and coagulation system activation are the main pathological bases of MODS. In recent years, many studies have indicated that systemic inflammatory response syndrome induced by inflammatory mediators, such as TNF-α, IL-10, IL-6, platelet activating factor and NO etc. are the key factors of MODS recurrence and development. The nuclear factor κB (NF-κB) was a ubiquitous nuclear transcription factor, which could regulate the transcriptions of TNF-α, IL-10, IL-6 and other cytokines as well as intercellular adhesion molecule[3-5]. So far, there was no effective method to prevent the development of MODS and there was no specific therapeutic measure in clinical practice. MODS traditional treatment methods generally include fasting and water electrolyte balance, gastrointestinal decompres-

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sion, controlling infection, etiological treatment, organ function protection and nutrition support, etc. However, their efficacies were not satisfactory and the effective rate was less than 50%[6]. Hemodialysis (HD) combined with hemoperfusion (HP) obtained the remarkable effects in eliminating exogenous and endogenous imune inflammatory substances and treating water, electrolyte and acid-base balance disorders through the absorption of hemoperfusion apparatus through high throughput filter as well as supporting organ function[7]. So it has been used in the treatment of MODS. In this study, HD+HP combined therapy was used for MODS patients, and we observed the effects of HD+HP combined therapy on peripheral inflammatory cytokines and NF-κB.

Patients and Methods

General data

92 cases of MODS patients undergoing hemodialysis in our hospital from December 2012 to December 2014 were randomly divided into the control group and observation group, with 46 cases in each group. There were 30 cases of male and 16 cases of female in the control group, aged 31-78 years old, averagely aged (43.67±13.52) years; including 9 cases of multiple trauma, 7 cases of simple cerebral trauma, 6 cases of severe traffic accident, 4 cases of cardio-pulmonary resuscitation, 4 cases of extensive burn, 4 cases of diffuse peritonitis severe pancreatitis, 3 cases of severe myocarditis, 3 cases of septic shock, drug and toxicant toxication. There were 32 cases in the observation group, including 32 cases of male and 14 cases of female, aged 33-78 years old, averaged aged (42.32±12.92) years, including 10 cases of multiple trauma, 8 cases of simple cerebral injury, 7 cases of serious traffic accident, 5 cases of cardio-pulmonary resuscitation, 3 cases of extensive burn, 3 cases of diffuse peritonitis severe pancreatitis, 2 cases of septic shock, 3 cases of drug and toxicant toxication. There was no statistical significance in gender, age or disease type in all patients (p>0.05). This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Xinxiang Medical University. Written informed consent was obtained from all participants.

Treatment method

The patients in two groups were treated with folic acid, ferralia, calcium carbonate, alimentary control, erythropoietin (EPO) and other basic treatments. The patients combined with infection were treated with antibiotics. The patients combined with diabetes mellitus were treated with insulin to control blood sugar. The patients combined with hypertension were treated with hypotensive drugs to control blood pressure. The patients in the control group were treated with HD. The bicarbonate was taken as the dialysate. The dialysate flow was 500 ml/min. The blood flow velocity was 250 ml. The ultrafiltration coefficient was 5.5 mL (h·mmHg). The each dialysis time was 4h. The dialysis was conducted 3 times each week. Low-molecular weight heparin was used for anticoagulation. The internal arteriovenous fistula was used. On the basis of the control group, hemoperfusion was carried out once a week in the observation group. Adsorption column heparinization was conducted according to the disposable hemoperfusion apparatus (Jianfan Biotechnology Co., Zhuhai, China). The hemoperfusion apparatus was connected in front of the hemodialyzer (Fresenius Kabi, Bad Homburg, Germany) in series. 2 L heparin normal saline (20 mg/L) was prepared. The pipeline and hemoperfusion apparatus were flushed. The hemoperfusion apparatus was slapped gently with hands to remove the bubbles and particles. The treatment time was 2 h. The first dose of heparin was 0.6-0.8 mg/kg and was superadded to 10-14 mg/kg. The hemoperfusion apparatus was removed. The hemodialysis was continued for 2 h. There were a total of 4 h. The patients in two groups were treated for 3 months.

Levels of Scr, BUN, TC and Alb in two groups of patients before and after treatment

2 ml fasting peripheral venous blood in patients of two groups was extracted before and after treatment. The levels of serum creatinine (Scr), total cholesterol (TC), blood urea nitrogen (BUN) and serum albumin (ALB) were detected by the fully automatic biochemical analyzer (Beckman-Coulter, Miami, FL, USA).

Western blot

The PBMC in peripheral blood was obtained by isolating human lymphocyte separation medium (Dingguo Biotechnology Co., Beijing, China) in two groups before and after treatment. The plasmosin and nucleoprotein were obtained from PBMC according to the cytoplasm extraction kit (Dingguo Biotechnology Co., Beijing, China).

The protein concentration was determined using BCA protein detection kit (Beyotime Biotechnology Co., Shanghai, China). 4* loading buffer was added and boiled for 10 min; SDS-PAGE was conducted. The protein was transferred to PVDF membrane and 5% skim milk powder was used to close for 30 min. The mouse-anti-human NK-κB p65 polyclonal antibody (Roche, Basel, Switzerland) (1:1000 dilution) was added, incubated overnight at 4°C and washed with PBST for 3 times. The rabbit-anti-mouse monoclonal antibody (Roche, Basel, Switzerland) was added and incubated for 1 h and colorated with DAB, photograhed. The β-actin was taken as the internal reference for cytoplasm. The Lamin B1 was taken as the internal reference for nucleus.

ELISA analysis

2 ml fasting peripheral venous blood was extracted respectively from two groups before treatment and after treatment, placed at room temperature for 30 min; centrifuged by 4000 rpm for 10 min. The supernatant was subpackaged in 1.5 ml EP tube. The levels of TNF-α and IL-6 strict were detected strictly according to ELISA kit (Boster Inc., Wuhan, China). Three complex holes were set for each sample and standard. The OD value was measured at 492 nm using the microplate reader (Thermo Scientific, Rockford, IL, USA).

Statistical Analysis

All data were analyzed using SPSS 17.0 software (SPSS Inc, Chicago, IL, USA). The measurement data were expressed using X±S. The measurement data among multiple groups were analyzed using ANOVA. The pairwise comparison was performed using LSD method. $p<0.05$ showed that the difference was statistically significant.

Results

Comparison of Scr, BUN, TC, Scr and ALB in two groups before and after treatment

There was no statistical significance in all indicators between the control group and observation group before treatment ($p>0.05$). The levels of Scr, BUN and TC were significantly decreased after treatment ($p<0.05$), the level of Alb was significantly increased ($p<0.05$). The indicators showed statistically significance in all indicators improvement in the observation group ($p<0.05$) (Figure 1).

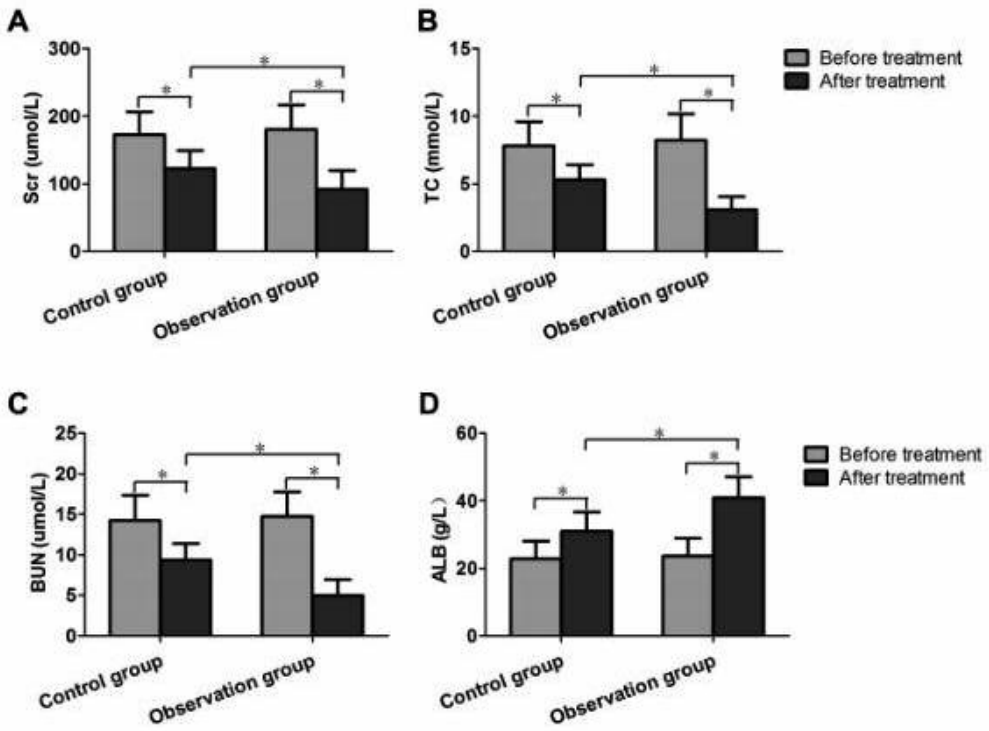

Figure 1. Comparison of Scr, BUN, TC, Scr and Alb in two groups before and after treatment. **A,** Comparison of Scr level; **B,** Comparison of TC level; **C,** Comparison of BUN level; **D,** Comparison of Alb level.

Comparison of NK-κB p65 protein levels in two groups before and after treatment

The Western blot analysis result of NK-κB p65 protein in peripheral white blood cell in two groups of patients before and after treatment was shown in Figure 2. There was no statistical significance in NK-κB p65 protein in peripheral white blood cell before treatment ($p>0.05$). NK-κB p65 protein in peripheral white blood cell was significantly increased in the observation group after treatment compared with the control group ($p<0.05$). The NK-κB p65 protein in nucleus was significantly decreased and the difference was statistically significant ($p<0.05$).

Comparison of TNF-α and IL-6 protein levels in two groups before and after treatment

Before treatment, the levels of serum TNF-α and IL-6 were not significantly different between the control group and observation group ($p>0.05$); after treatment, compared with the control group, the levels of TNF-α and IL-6 were significantly decreased and the difference was statistically significant ($p<0.05$) (Figure 3).

Discussion

MODS is still an unpredictable serious complication of many critical diseases at end stage. The mortality is still about 60-80% even if it is positively treated. It is one of the important causes of death in critically ill patients and its threat to human health is becoming more and more prominent[8]. In recent years, the pathogenesis of MODS has been studied. It has been clear that the infective or non-infective factors act on the body, which causes the increased inflammatory mediators or cytokines, resulting in systemic inflammatory response syndrome (SIRS), and then damage many organs, resulting in MODS. The existing researches confirmed that SIRS was the fundamental cause of MODS. To block SIRS brought the breakthrough progress for the treatment of MODS. So far, the mediators directly involving in inflammatory reaction include TNF-α, IL-6, IL-1, IL-8, platelet activating factor (PAF) and myocardial inhibiting factor, etc.[9-11]. The levels of TNF-α and IL-6 in peripheral blood of MODS patients were detected using ELISA method. The result showed that the levels of TNF-α and IL-6 were significantly higher than normal values, and the body was in a state of aggregation of inflammatory factors.

In addition, many research results showed that NK-κB, as an important transcription factor mediating the inflammatory mediators, is the final confluence of the inflammatory signals, which plays an important role in regulating immune and inflammatory responses, and may be in the central position in MODS[12]. NK-κB can transmit the endonuclear and extranuclear information, plays an important role in immune, stress response, inflammation and cell apoptosis. In the static state, NK-κB in the cytoplasm was connected through IκB and appeared to inactive homodimer or heterodimer[13-15]. When the body was stimulated by pathological stimuli so as to activate inflammatory and other signal transduction pathways, IκB kinase in cytoplasm and nucleus in peripheral blood

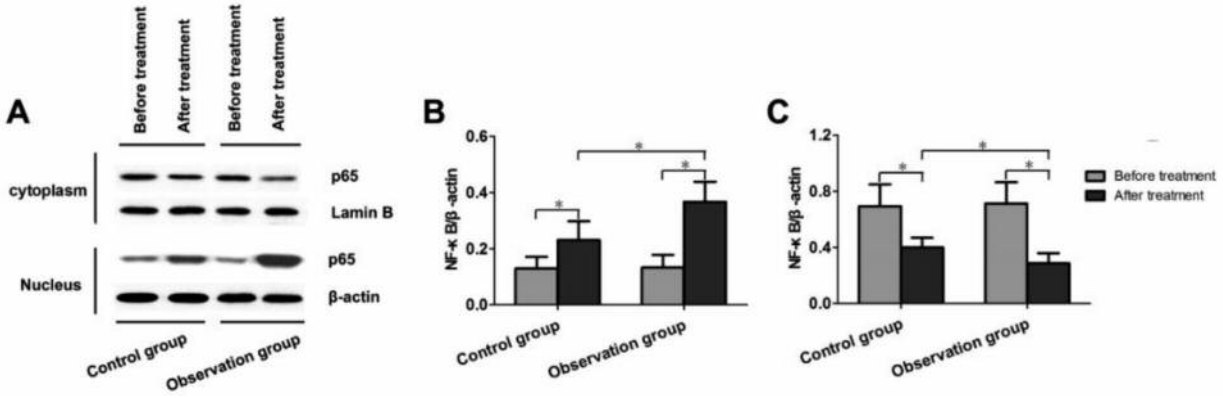

Figure 2. Comparison of NK-κB p65 protein levels in two groups before and after treatment. **A,** Western blot showed NF-κB p65 of white blood cells; **B,** Quantitative analysis showed NF-κB p65 of white blood cells in cytoplasm; **C,** Quantitative analysis showed NF-κB p65 of white blood cells in nucleus.

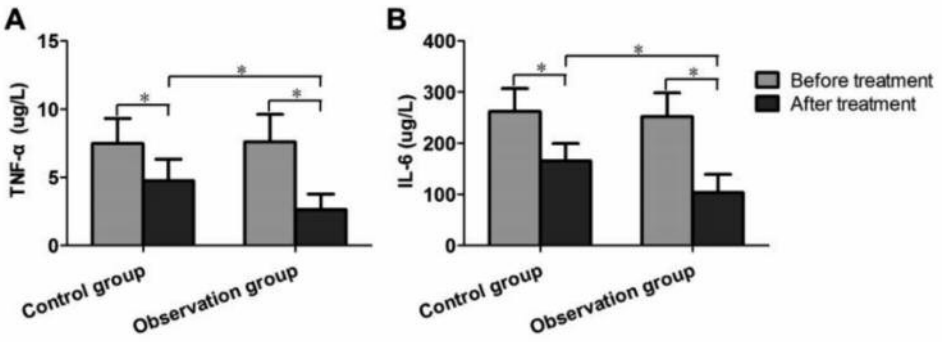

Figure 3. Comparison of TNF-α and IL-6 protein levels in two groups before and after treatment. ***A,*** TNF-α level of peripheral blood in two groups; ***B,*** IL-6 level of peripheral blood in two groups.

of MODS patients was detected using Western blot method. The result showed that the level of NF-κB in cytoplasm of MODS patients was significantly lower than the normal value, the level of NF-κB in nucleus was significantly higher than the normal value. Combined with the levels of TNF-α and IL-6 in peripheral blood of MODS patients, the result confirmed that NF-κB occurred to nuclear translocation in the occurrence and development of MODS, promote the expression of inflammatory factors.

Hemoperfusion (HP) is a blood purification method which can eliminate various inflammatory mediators, cytokines and vasoactive substances and endotoxin etc. through dispersion or convection current to produce adsorption and filtration, which can maintain the balance of water and electrolyte, persistently and stably regulate azotemia, maintain the stability of blood purification, and transfused the purified blood in body. HP can maintain the stability of cardiovascular function so as to delay MODS caused by these inflammatory factors[16-18]. In this study, compared with simple HD treatment, the level of NK-κB p65 protein in cytoplasm of MODS patients was significantly higher after (HP+HD) combined therapy, while the level of NK-κB p65 protein was significantly decreased, the levels of inflammatory cytokines IL-6 and TNF-α in peripheral blood were decreased significantly. The study showed that there were high catabolism and severe internal environment disturbance in MODS patients. The levels of BUN and Scr in peripheral blood were gradually decreased, the difference was statistically significant before and after treatment. And the decrease in (HP+HD) combined therapy group was more significant ($p<0.05$), indicating that HP+HD could improve blood biochemical parameters, adjust water electrolyte and acid-base disorder, improve the internal environment, which could be used safely and was suitable for the treatment of critical disease. Moreover, compared with simple HD therapy, HP+HD combined therapy was more accorded with physiological status, the haemodynamics was more stable, the solute clearance rate was higher, could provide sufficient nutrition, the liquid could be well controlled.

Conclusions

HP+HD combined therapy in the treatment of MODS patients can significantly improve various biochemical indicators, effectively remove the inflammatory mediators, reduce the inflammatory reaction of patients through inhibiting the activation level of NK-κB, which has significant clinical effect.

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Conflict of interest

The Authors declare that they have no conflict of interests.

References

1. Ramírez M. Multiple organ dysfunction syndrome. Curr Probl Pediatr Adolesc Health Care 2013; 43: 273-277.
2. Nguyen TC, Cruz MA, Carcillo JA. Thrombocytopenia-associated multiple organ failure and acute kidney injury. Crit Care Clin 2015; 31: 661-674.

3. Li CC, Lan HX. Analysis of risk factors and early nursing intervention for 77 cases multiple organ dysfunction syndrome. The Journal of Practical Medicine 2009; 25: 2955-2958. In Chinese
4. Asserraji M, Benameur I, Maoujoud O, El Kharras A, Hajbi H, Filali K. Late care in marathon runs leading to exertional heat stroke with multiple organ failure. Asian J Sports Med 2014; 5: 136-138.
5. Teoh H, Quan A, Creighton AK, Annie Bang KW, Singh KK, Shukla PC, Gupta N, Pan Y, Lovren F, Leong-Poi H, Al-Omran M, Verma S. BRCA1 gene therapy reduces systemic inflammatory response and multiple organ failure and improves survival in experimental sepsis. Gene Ther 2013; 20: 51-61.
6. Panteli ES, Fligou F, Aretha D, Karamanakos PN. Role of serotonin in MODS: deficiency of serotonin protects against zymosan-induced multiple organ failure in mice. Shock 2015; 44: 383.
7. Li DY, Chen YG, Li YM. Effect hemodialysis and hemoperfusion combined therapy on micro-inflammatory factors and calcium phosphorus metabolism of patients with long-term hemodialysis. Medical Recapitulate 2013; 19: 1493-1496.
8. Kumar A, Chari ST, Vege SS. Can the time course of systemic inflammatory response syndrome score predict future organ failure in acute pancreatitis? Pancreas 2014; 43: 1101-1105.
9. Pang T, Chen W, Lu ZM, Luo TH, Zhou H, Xue XC, Bi JW, Fang GE. Endothelial progenitor cells are influenced by serum of patients with systemic inflammatory response syndrome or multiple organ dysfunction. Eur Rev Med Pharmacol Sci 2013; 17: 3169-3177.
10. Volpin G, Cohen M, Assaf M, Meir T, Katz R, Pollack S. Cytokine levels (IL-4, IL-6, IL-8 and TGFβ) as potential biomarkers of systemic inflammatory response in trauma patients. Int Orthop 2014; 38: 1303-1309.
11. Li A, Xiong J, Chen Z. IL-6, TNF-α, and iNOS is associated with decreased colonic contraction in rats with multiple organ dysfunction syndrome. J Surg Res 2012; 178: e51-57.
12. Nakajima S, Chi Y, Gao K, Kono K, Yao J. eIF2α-Independent inhibition of TNF-α-triggered NF-κB activation by salubrinal. Biol Pharm Bull 2015; 38: 1368-1374.
13. Linkermann A, Stockwell BR, Krautwald S, Anders HJ. Regulated cell death and inflammation: an auto-amplification loop causes organ failure. Nat Rev Immunol 2014; 14:759-767.
14. Michelena J, Altamirano J, Abraldes JG, Affò S, Morales-Ibanez O, Sancho-Bru P, Dominguez M, García-Pagán JC, Fernández J, Arroyo V, Ginès P, Louvet A, Mathurin P, Mehal WZ, Caballería J, Bataller R. Systemic inflammatory response and serum lipopolysaccharide levels predict multiple organ failure and death in alcoholic hepatitis. Hepatology 2015; 62: 762-772.
15. Li N, Hu X, Liu Y, Wang Y, Wang Y, Liu J, Cai W, Bai X, Zhu X, Han J, Hu D. Systemic inflammatory responses and multiple organ dysfunction syndrome following skin burn wound and Pseudomonas aeruginosa infection in mice. Shock 2013; 40: 152-159.
16. Sadaka F, Aggu-Sher R, Krause K, O'Brien J, Armbrecht ES, Taylor RW. The effect of red blood cell transfusion on tissue oxygenation and microcirculation in severe septic patients. Ann Intensive Care 2011; 1: 46.
17. Hirabayashi K, Shiohara M, Saito S, Tanaka M, Yanagisawa R, Tsuruta G, Fukuyama T, Hidaka Y, Nakazawa Y, Shimizu T, Sakashita K, Koike K. Polymyxin-direct hemoperfusion for sepsis-induced multiple organ failure. Pediatr Blood Cancer 2010; 55: 202-205.
18. Trzeciak S, McCoy JV, Phillip Dellinger R, Arnold RC, Rizzuto M, Abate NL, Shapiro NI, Parrillo JE, Hollenberg SM; Microcirculatory Alterations in Resuscitation and Shock (MARS) investigators. Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis. Intensive Care Med 2008; 34: 2210-2217.