

Effects of HFHD on oxidative stress, micro-inflammatory state and cellular immune function in patients with diabetic nephropathy and hemodialysis.

Junyue Huang, Shouyan Lu*

Department of Nephrology, Gansu Provincial Hospital, Lanzhou, Gansu, PR China

Abstract

Objective: To investigate the effects of High Flux Hemodialysis (HFHD) on oxidative stress, micro-inflammatory state and cellular immune function in patients with diabetic nephropathy and hemodialysis.

Methods: From March 2016 to March 2017, 90 Elderly Patients with Diabetic Nephropathy and Hemodialysis in our hospital were selected as the research objects and divided into group A and B according to the different treatment, with 45 cases in each group. HFHD was performed in group A while Low Flux Hemodialysis (LDHD) was performed in group B. Before and after treatment, the expression levels of Superoxide Dismutase (SOD), Glutathione Peroxidase (GSHPx), Interleukin-6 (IL-6), Tumor Necrosis Factor alpha (TNF- α), High Sensitive C-reactive protein (HS-CRP) and the changes of peripheral blood T lymphocyte subsets were checked and compared between those two groups.

Results: Before treatment, there was no significant difference in serum oxidative stress factors of SOD and GSHPx between the two groups. After treatment, the levels of serum oxidative stress factors of SOD and GSHPx in group A were increased significantly and higher than those in group B. Before treatment, no significant differences of serum micro-inflammatory factors of IL-6, TNF- α , hs-CRP were found between the two groups. After treatment, however, the expression levels of IL-6, TNF- α , hs-CRP were significantly reduced and lower than those in group B. Before treatment, no significant differences of peripheral blood T lymphocyte subsets of CD3⁺, CD4⁺, CD8⁺, CD3⁺/CD8⁺ were found between the two groups. After treatment, the expression of CD3⁺, CD4⁺ and CD4⁺/CD8⁺ in group A was significantly increased and higher than that in group B. However, there was no significant difference in the expression of CD8⁺ between the two groups.

Conclusion: The implementation of HFHD treatment in elderly patients with Diabetic Nephropathy and Hemodialysis could effectively improve the oxidative stress, micro-inflammatory state and cellular immune function. It had great value and was worthy of popularization and application.

Keywords: Elderly patients, Diabetic nephropathy, High flux hemodialysis, Inflammation, Oxidative stress, Cellular immunity.

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Introduction

The prevalence rate of end-stage diabetic nephropathy is obviously increasing in the world, with many complications and high mortality. At present, Hemodialysis (HD) and Continuous Ambulatory Peritoneal Dialysis (CAPD) are still the alternative therapies for end-stage diabetic nephropathy [1]. Previous study shows that abnormal immune function accompanied with abnormal expression of inflammatory factors is found in patients with end-stage diabetic nephropathy, and long-term hemodialysis treatment exacerbates inflammatory reaction and oxidative stress, to promote the occurrence of complications such as atherosclerosis, malnutrition, heart cerebrovascular diseases [2]. Therefore, there are obvious immune hypofunction, micro inflammation and oxidative stress in patients with end-stage

renal disease, which will aggravate the complications and even lead to poor prognosis. Decreased levels of micro-inflammatory markers of hs-CRP and IL-6 and oxidative stress markers of SOD and GSHPx, are closely related to the occurrence of complications such as cardiovascular events and adverse outcomes [3]. However, there is still no effective treatment for improving the micro inflammation, oxidative stress and immune function in hemodialysis patients with diabetic nephropathy. High Flux Hemodialysis (HFHD) is an efficient method of blood purification. It has been found to reduce the incidence of cardiovascular and cerebrovascular events and mortality in hemodialysis patients effectively [4,5]. At present, there are few researches on the effects of HFHD on oxidative stress, micro-inflammatory state and cellular immune function in elderly patients with diabetic nephropathy

undergoing hemodialysis. In view of this, this study was conducted to analyse the serum inflammatory factors, oxidative stress and peripheral blood T lymphocyte subsets in diabetic nephropathy hemodialysis elderly patients with HFHD treatment.

Materials and Methods

Clinical data

From March 2016 to March 2017, 90 Elderly Patients with Diabetic Nephropathy and Hemodialysis in our hospital were selected as the research objects. Among them, 47 males, 43 females, aged 60 to 74 y, average age of 65.7 ± 1.2 y. Inclusion criteria: $\text{Scr} > 707$ mol/L or $\text{Ccr} < 10$ min/ml, receiving maintenance hemodialysis for more than 6 months. Exclusion criteria: no serious infections, blood diseases, malignant tumor, liver disease, mental illness or serious cardiovascular and cerebrovascular diseases; blood pressure control was stable, and no surgery, radiotherapy or chemotherapy, without the use of other immunosuppressive agents during the study period, without blood transfusion. Patients in two groups were given dialysis 3 times a week, 4 h each time. Patients were divided into group A and B according to the different treatment, with 45 cases in each group. HFHD was performed in group A while Low Flux Hemodialysis (LDHD) was performed in group B. There was no significant difference in age, sex ratio or dialysis time between the two groups.

Methods

Patients in two groups were dialysis using Amartya Sen, 4008 S hemodialysis machine, Germany. Patients in group A were treated with HFHD, using dialyzer FX80 dialyzer, dialysate flow rate 800 ml/min, blood flow 200-250 ml/min, dialysis frequency 3 times/w, 4 h/time. Patients in group B were treated with FX80 dialyzer. Both the two groups were treated for 6 months.

Detection indexes

Before and after treatment, 5 ml of elbow vein blood was collected, and the upper serum was retained. Serum expression levels of IL-6 and TNF- α were detected by ELISA, and SOD, GSHPx and HS-CRP were detected by immune transmission turbidimetry. The kits were purchased from R and D Company of the United States. CD3^+ , CD4^+ , CD8^+ , $\text{CD4}^+/\text{CD8}^+$ cells

were detected by flow cytometry (BD FACSCalibur, United states). Strictly follow the operating instructions of kit instructions.

Statistical methods

The data were analysed with SPSS21.0 software. The measurement data were expressed as mean \pm standard deviation and the t-test were used. Chi-square test was used to compare the categorical data. $P < 0.05$ suggests the difference is statistically significant.

Results

Comparison of oxidative stress factors before and after treatment in two groups

Before treatment, there was no significant difference in serum oxidative stress factors of SOD and GSHPx between the two groups. After treatment, the levels of serum oxidative stress factors of SOD and GSHPx in group A were increased significantly and higher than those in group B (Table 1).

Table 1. Comparison of oxidative stress factors before and after treatment in two groups.

Group	n	SOD ($\mu\text{U/L}$)		GSHPx (U/L)	
		Before treatment	After treatment	Before treatment	After treatment
Group A	45	56.72 ± 7.85	69.51 ± 6.92	183.26 ± 15.93	239.55 ± 25.48
Group B	45	56.68 ± 7.33	58.42 ± 5.73	183.21 ± 16.06	190.45 ± 21.17
t		1.045	8.167	0.723	6.106
P		0.562	0.016	0.648	0.027

Comparison of micro inflammatory factors before and after treatment in two groups

Before treatment, no significant differences of serum micro-inflammatory factors of IL-6, TNF- α , hs-CRP were found between the two groups. After treatment, the expression levels of IL-6, TNF- α , hs-CRP were significantly reduced and lower than those in group B (Table 2).

Table 2. Comparison of micro inflammatory factors before and after treatment in two groups.

Group	n	IL-6 ($\mu\text{g/L}$)		TNF- α (ng/L)		hs-CRP (mg/L)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Group A	45	22.12 ± 2.36	12.78 ± 1.24	60.33 ± 6.75	49.81 ± 5.04	9.17 ± 0.85	6.8 ± 0.46
Group B	45	22.09 ± 2.54	19.46 ± 1.32	60.26 ± 5.82	56.02 ± 6.13	9.15 ± 0.93	8.5 ± 0.37
t		0.955	9.047	0.604	7.886	0.792	6.153

p	0.161	0.013	0.472	0.022	0.304	0.028
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Comparison of the changes of peripheral blood T lymphocyte subsets before and after treatment in two groups

Before treatment, no significant differences of peripheral blood T lymphocyte subsets of CD3⁺, CD4⁺, CD8⁺, CD4⁺/CD8⁺

were found between the two groups. After treatment, the expression of CD3⁺, CD4⁺ and CD4⁺/CD8⁺ in group A was significantly increased and higher than that in group B (P<0.05). However, there was no significant difference in the expression of CD8⁺ between the two groups (Table 3).

Table 3. Comparison of the changes of peripheral blood T lymphocyte subsets before and after treatment in two groups.

Group	n	CD3 ⁺ (%)		CD4 ⁺ (%)		CD8 ⁺ (%)		CD4 ⁺ /CD8 ⁺ (%)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Group A	45	32.67 ± 3.28	41.95 ± 4.02	30.22 ± 4.04	40.62 ± 5.28	25.04 ± 3.23	24.62 ± 3.38	1.14 ± 0.23	1.52 ± 0.14
Group B	45	32.71 ± 3.41	38.14 ± 3.38	30.31 ± 3.96	36.32 ± 4.87	25.02 ± 2.75	26.21 ± 3.16	1.13 ± 0.18	1.44 ± 0.16
t		0.135	5.092	0.602	6.194	0.441	1.239	0.532	6.008
p		0.647	0.036	0.543	0.029	0.603	0.072	0.549	0.031

Discussion

With the continuous improvement and development of hemodialysis technology, the survival time of patients with hemodialysis has been greatly improved. However, the quality of life of these patients is still a problem and the incidence of cardiovascular complications is high [6]. In the United States and other economically developed western countries, the annual mortality rate of hemodialysis patients is still up to 20%-30% [7]. The proportion of elderly patients with diabetic nephropathy hemodialysis in dialysis patients increased year by year. In clinical practice, it is found that elderly patients with diabetic nephropathy hemodialysis have worse prognosis and higher risk of death. The main reason is that in elderly patients with diabetic nephropathy and hemodialysis, there are self-metabolic disorder due to uremia, biological incompatibility of dialysis membranes, inadequate clearance of inflammatory mediators, antioxidants lost from dialysis solutions and low immune function which make the body chronically in the state of micro inflammation and oxidative stress reaction. Among them, oxidative stress and micro inflammatory state interact with each other and promote each other. The increase of lipid peroxidation, the decrease of antioxidant substances and the decrease of antioxidant capacity cause the patients to be in the state of oxidative stress reaction. At the same time, inflammatory factors activated by micro-inflammatory state can also amplify oxidative stress response through the oxidation of nicotinamide adenine dinucleotide phosphate oxidase complex on the white blood cell membrane. And oxidative stress further induces the formation of inflammatory mediators and growth factors, resulting in a vicious cycle [8]. Such state can lead to severe complications such as atherosclerosis, malnutrition, cardiovascular and cerebrovascular diseases, and it is the main risk factor for high mortality and hospitalization rate in elderly patients with diabetic nephropathy and hemodialysis. Hs-CRP is one of the

major components of acute phase response proteins, and can be used as the most sensitive and specific indicator of low level inflammation. The level of hs-CRP exceeding normal (>5-10 mg/L) can be diagnosed as micro-inflammatory state [9]. Hs-CRP levels are higher in hemodialysis patients than in healthy subjects, and are associated with increased cardiovascular events and mortality in dialysis patients [10]. IL-6 and TNF-α are important proinflammatory cytokines, which in addition to acting directly on the tissue cells, can also activate other inflammatory mediators to play a role in mesangial cell proliferation, sclerosis and deterioration of renal disease. SOD is an important enzyme to scavenge oxygen free radicals *in vivo*, which can promote the production of lipid peroxidation chain reaction and protect the cells from free radical damage. GSHPx is an important peroxidase decomposing enzyme widely existing *in vivo*, which can reduce peroxides to hydroxyl compounds, and protect the integrity of cell membrane structure and function. The decreased levels of SOD and GSHPx suggest a decrease in antioxidant capacity [11]. In elderly patients with diabetic nephropathy and hemodialysis, there are micro inflammation and oxidative stress state regardless of dialysis treatment. In this study, IL-6, TNF-α and hs-CRP were used as indicators for the detection of micro-inflammation, and SOD and GSHPx were indicators of oxidative stress. The results showed that the markers of oxidative stress and inflammatory increased in the two groups before treatment, suggesting the patients were in oxidative stress and inflammatory state.

HFHD is an efficient blood purification method. It is a technique of conventional hemodialysis using a high-volume blood filter on a volume controlled hemodialysis machine. Unlike LFHD, which scavenges toxins by dispersion, HFHD can remove solutes through 3 ways: dispersion, convection and adsorption. Because of its high diffusion performance and hydraulic permeability, the polymer membrane can obviously

remove the solutes in the medium and large molecules based on the removal of small solutes [12]. Conventional hemodialysis can't improve the micro-inflammatory state of patients with hemodialysis, mainly because the molecular weight of inflammatory factors is so large that can't be filtered out by the conventional low flux dialyzer due to pore size limit. Compared with the low flux dialyzer, the high flux dialyzer also has good biocompatibility [13]. It can reduce the complement activation and inflammatory reaction of the patients, at the same time, it can improve the hematopoietic function of the bone marrow. High flux dialyzer can reduce the medium and large molecular levels of toxins, remove a variety of medium inflammation molecules *in vivo*, improve the dialysis patients with chronic micro-inflammatory state and malnutrition, and reduce cardiovascular and cerebrovascular events of death. The European MPO study also showed that the risk of death was reduced in patients treated with HFHD in the population of serum albumin less than 40 g/L or diabetes, and diabetes patients with serum albumin less than 40 g/L benefited more [14]. However, there are few reports about whether HFHD can improve the oxidative stress and micro-inflammatory state in elderly patients with diabetic nephropathy and hemodialysis. In present study, 6 months after treatment, the serum levels of IL-6, TNF- α and hs-CRP in elderly patients with diabetic nephropathy and hemodialysis treated with HFHD were significantly lower, while the levels of serum SOD and GSHPx were significantly higher than patients treated with LFHD. The results confirmed that HFHD had the effect of improving oxidative stress and micro-inflammatory state in elderly patients with diabetic nephropathy and hemodialysis. The mechanisms may be as follows: (1) improving insulin resistance, inhibiting the high expression of IL-6 and TNF- α , and reducing the increase of hs-CRP synthesis; (2) directly removing IL-6, TNF- α , hs-CRP and other large and medium molecular toxins; (3) the dialysis membrane with good biocompatibility can reduce the complement activation and inflammatory reaction of patients, and reduce the endotoxin reverse ultrafiltration; (4) regulating the disorder of lipid metabolism, improving the metabolism of calcium and phosphorus, and decreasing the level of serum parathyroid hormone.

So far, the mechanism of immune dysfunction in elderly patients with diabetic nephropathy and hemodialysis has not been clear. It is generally believed that the accumulation of uremic toxins in the blood is an important factor in inhibiting the immune function of T lymphocytes. The frequent incidence of bacteria, tuberculosis infection and high incidence of cancer in elderly patients with diabetic nephropathy hemodialysis are related to immune dysfunction, which is characterized by T lymphocyte predominant cellular immune dysfunction [15]. T lymphocytes are important immune cell populations. Under physiological condition, the coordination of each subpopulation of cells *in vivo* produces a moderate immune response, which not only eliminates the foreign body, but also does not damage the body's own tissues. When the number and function of T lymphocyte subsets are abnormal, it leads to immune disorders. CD4⁺ is an auxiliary T lymphocyte marker

and has the effect of enhancing immune response; CD8⁺ is an inhibitor marker of cytotoxic T cells and has immunosuppressive effect. The decrease of lymphocyte subsets CD4⁺/CD8⁺ ratio is closely related to the severity of the disease and poor prognosis. The levels of CD3⁺, CD4⁺ and CD4⁺/CD8⁺ in patients with diabetic nephropathy are decreased, and the level of CD8⁺ is elevated, which means the cellular immune function has been destroyed, and the immune function is low and easy to be complicated with infection [16]. In present study, 6 months after treatment, the levels of CD3⁺, CD4⁺ and CD4⁺/CD8⁺ in elderly patients with diabetic nephropathy and hemodialysis treated with HFHD were significantly increased and were higher than patients treated with LFHD. This suggested that the cellular immune function of elderly patients with diabetic nephropathy and hemodialysis could be improved significantly after HFHD dialysis. The possible reason is that LFHD mainly relies on dispersion and has good scavenging effect on small molecular hydrophilic toxins. However, LFHD has poor scavenging effect of macromolecules uremic toxins, which accumulate in the body and lead to impaired immune function.

At present, the treatment of micro-inflammation, oxidative stress and cellular immune dysfunction in elderly patients with diabetic nephropathy and hemodialysis is still immature, and the prevention and treatment methods are limited. HFHD is the development direction of conventional hemodialysis which has the advantage of reducing the complications caused by long-term dialysis and improving the quality of life and survival rate of patients. Our study showed that the implementation of HFHD treatment in elderly patients with diabetic nephropathy and hemodialysis could effectively improve the oxidative stress, micro-inflammatory state and cellular immune function. Of course, this research also had many shortcomings. For example, the sample size was small, without considering the effect of dialysate purity on oxidative stress, micro-inflammation, cellular immune dysfunction, no observation of HFHD on cardiovascular disease associated protein binding toxins clearance, which would be gradually improved in the future research. At the same time, HFHD also has some defects. Due to the larger ultrafiltration coefficient, more albumin may be lost, and the risk of reverse ultrafiltration exists. So large sample and multicenter clinical studies should be conducted in the future.

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***Correspondence to**

Shouyan Lu

Department of Nephrology

Gansu Provincial Hospital

PR China