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The Effect of Hemodialysis on the Expression of CXCL8 and its mRNA in Neutrophils of the Patients with Chronic Renal Failure

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ABSTRACT

CXCL8 may involve in a variety of inflammatory and immune response. To investigate the effect of hemodialysis on the expression of CXCL8 in serum and its mRNA in neutrophils of the patients with chronic renal failure (CRF). It was used to dynamically observe of Hemodialysis on the concentration of the CXCL8 in serum and the levels of CXCL8 mRNA in neutrophils were respectively measured by ELISA and PCR. The house keeping gene encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal reference, the ratio of lgcDNA/lgGAPDH was regarded as the extreme level of CXCL8 mRNA. The serum level of CXCL8 and expression of CXCL8 mRNA in neutrophils of the chronic renal failure were (128.892±17.101) pg/mL and (1.332±0.138) lgcDNA/lgGAPDH. There was no significant difference compared with the normal controls (t=2.8468, P=0.062; t=1.953, P=0.0559). Further observation showed that the levels of CXCL8 and mRNA in neutrophils of the patients with infection were (136.048±15.551) pg/mL and (1.391±0.119) lgcDNA/lgGAPDH, and were significantly higher than those in the cases without infection (t=3.2901, P= 0.023; t=3.3701, P=0.0018). After treatment of Hemodialysis for 12h, CXCL8 and mRNA levels in peripheral blood of the patients were decreased very rapidly, and mainly found in the patients with infection. There was no significant difference between the patients with and without infection (t=0.936, P=0.3468; t=1.7741, P=0.0848). Follow up observation for 48 h after Hemodialysis treatments, the levels of CXCL8 and mRNA in peripheral blood of the patients have a certain degree of up-regulation, but there was still no significant difference between the patients with and without infection (t=1.6975, P=0.0985; t=2.0575, P=0.472). CXCL8 can be as a sensitive indicator of the immunological reaction of micro inflammation in the patients, and tightly correlated with the merger of infection. The high concentration of CXCL8 in serum can be quickly filtered out by Hemodialysis in the short term and the transcription level of CXCL8 mRNA in neutrophils also be induced down regulation, and subsequently promote the recovery of injury via the inhibition pathway of micro inflammatory immune. In order to prevent excessive micro inflammation, the patients with infection should have been repeated Hemodialysis.

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Authors' Contribution
JW and ZL designed the study, performed the experiments and wrote the article. SX, XL and WC participated in the collection and analysis of the data. All authors contributed to the design and interpretation of the study.

Key words
Hemodialysis, Chronic renal failure (CRF), Neutrophils, CXCL8, Micro inflammatory reaction.

INTRODUCTION

Chronic renal failure (CRF) is now recognized as a global public health problem. About 8%~16% of the adult chronic kidney disease (CKD) has been confirmed to be further developed into renal failure (Stengel *et al*., 2014). It is defined as a series of symptoms and metabolic disorders caused by progressive and irreversible renal parenchymal injury, such as the reduction of glomerular filtration rate and the increase of urinary albumin excretion, retention of metabolites and toxic waste, disorder of hydroelectrolyte balance and acid-base (Jha and Prasad, 2016). When the progression of chronic kidney disease to kidney failure, a variety of metabolic dysfunction and the changes of microenvironment were cumulative *in vivo*, such as a large amount of extracellular matrix (ECM) accumulation in glomeruli, the proliferation of fibrous tissue around the glomerular, proliferation of mesangial cells, inflammatory infiltration of mononuclear macrophages and lymphocytes in the glomeruli and tubulointerstitium, so that the local inflammation was often repeatedly delayed. The immune complexes produced by some inflammatory reactions are deposited in the glomerular capillary loop, and trigger the complement system, and further destroy the podocyte lead to the damage of glomerular filtration membrane, and then

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lead to massive proteinuria (Vaziri *et al*., 2012).

CRF is a slow progressive pathological progress and its clinicopathologic characteristics are often associated with a certain degree of cellular immune function, for example, lymphatic tissue atrophy, lymphocytes decrease, so that the anti-infection ability in the host will be often reduced. It has been confirmed that the infection is a high risk factor for CRF, which can directly promote the pathological process of CRF (Rojas *et al*., 2013). CXCL8, an important cytokine or chemokine, can reflect the inflammatory state caused by infection, and may involve in a variety of inflammatory and immune response. It has been a hotspot of biological therapy and a novel strategy of targeted therapy in recent years (Nagy *et al*., 2016). In order to investigate the effects of hemodialysis on the clearance rate of CXCL8 and the inhibition of the inflammatory reaction, the typical CRF patients were selected and further dynamically observed in clinical trials, the detailed results were reported as follows.

MATERIALS AND METHODS

Clinical data

The thirty-seven patients (males 26, females 11, age from thirty-six to seventy-two years, average age 51.7±9.2 years) with chronic renal failure as outpatients at the Affiliated Hospital of Anhui University of Science and Technology (the First Peoples Hospital of Huainan), Huainan, China, from October 2014 to October 2013 were identified. The diagnosis of chronic renal failure was based on the criteria set out by the Chinese Medical Association. All patients underwent their family histories and clinical symptom inquiry, laboratory assessment (renal function examination, blood and urine routine examination) at their initial outpatient visit. All studied patients were negative for laboratory tests for heart disease, hepatitis, pneumonia, etc. No history of thyroid surgery. Twenty cases (males 12, females 8, age from twenty-nine to fifty-two years, average age 43.7±7.8 years) in the same period of the normal physical examination were regarded as normal controls. The study protocol was approved by the Ethics Committee of the Affiliated Hospital and Teaching Hospital of Anhui University of Science and Technology. All of the patients provided verbal permission for their data and records to be used in this study.

Reagents and instruments

The ELISA test kits used for the quantitative measurement of CXCL8 was purchased from DIACLONE, France. The fetal bovine serum was purchased from Bio Basic Inc, USA. The neutrophils isolation medium and whole blood red cells lysis buffer were all purchased from Tianjin HaoYang Biological Technology Co., Ltd. Hanks' balanced salts solution (HBSS), pH 7.2, with or without calcium and magnesium was purchased from Shenggong Biological engineering Limited by Share Ltd. Trizol reagent was purchased from Invitrogen Co., USA. RPMI 1640 complete culture medium was purchased from Sigma, USA. The extraction kits of the first strand of AMV cDNA were purchased from Shanghai Shenneng Gambling Co., China. Polymerase chain reaction (PCR) kits were purchased from Sangon Biotech (Shanghai) Co., China. The synthesis kits of script cDNA were purchased from Tiangeng Biotech (Beijing) Co., Ltd, China. LightCycler FastStart DNA Master SYBR Green I reagent was purchased from Roche Co., Germany. The automatic microplate washer (EXL-50X) and the automatic microplate-reader for ELISA (EXL-808) were all purchased from Bio-Tek Co., USA. The high-speed centrifuge (5415D) was purchased from Eppendorf® Co., Germany. The ultraviolet visible spectrophotometer (UV-5800PC) was purchased from Shanghai Element Analysis Instrument Co., China. The fluorescence microscope (Nikon E-400) was purchased from Nikon Co., Japan. Gradient PCR instrument (TP600) was purchased from TaKaRa Co., Japan. The iCyclez® real-time quantitative PCR instrument was purchased from Bio-Rad Co., USA. Molecular Imager ChemiDoc™ XRS$^+$ analysis system was purchased from Bio-Rad Co., USA.

Specimen collection

The total volume of 5 mL fresh peripheral venous blood from the patients was taken before breakfast, and distributed a sterile Eppendorf tube and an anticoagulant tube (heparin), respectively. The fresh heparinized blood was used to conventionally separate the neutrophils and further to prepare total RNA extraction, and to detect the mRNA transcript level of CXCL8. The other normal clean Eppendorf tube was used to routinely separate the plasma for detection of free CXCL8 concentration.

Detection of CXCL8 in serum/plasma

Solid phase sandwich ELISA method was used to detect in our studies. The standard curve was drawn by 1:2 dilution of the standard dilution solution provided by the reagent company. The peripheral venous blood of the cases was collected in batches and then used to isolate the fresh serum. The control groups of CXCL8 markers with two blank pores, two negative pores two positive pores were made in each test. Every titer was measured twice by ELISA analyzer at 450 nm and the final average OD of titer was then calculated. The positive threshold was ≥2.1, which was the rate of average OD of sample to the average titer OD of negative control. The minimum detectable dose

of human CXCL8 was usually less than the concentration of 19 pg/mL, and its detective value was range from 78 pg/mL to 5,000 pg/mL. The dilutions of the standard were taken as the abscissa and the OD data were taken as the ordinate in standard curves, so that the concentrations of CXCL8 in serum samples were calculated from these curves using the standard samples in the kits.

Extraction, isolation and purification of PBNs

After the volume of 2 mL heparin anticoagulant blood mixed with the equal volume Hank's liquid without Ca^{2+} and Mg^{2+}, and then layered the diluted blood over the surface of the PBNs isolation medium carefully. Conventionally centrifuge at 2500 rpm/min for 35 min at 20-25°C. The blood should separate out into six distinct bands, such as plasma, monocytes, mixed layer of neutrophil separation and PBNs, and the red blood cell pellet. Pipette the layer of PBNs and all of the isolation media beneath the PBNs carefully and the place the solution into another clean centrifuge tube. Take the diluted the PBNs solution to 5 mL with HBSS without Ca^{2+} and Mg^{2+} and then invert the tube several times to suspend the cells. The PBNs solution was centrifuged at 2000 rpm/min for 10 min so that a red pellet should be present at the bottom of the tube, containing large of PBNs and residual red blood cells. Remove the supernatant with a pipette carefully so that the pellet is not disturbed. Continue to centrifuge the tubes at 1500 rpm/min for 5 min and remove the supernatant with pipette as soon as possible. PBNs were resuspended in complete HBSS liquid (with Ca^{2+} and Mg^{2+}) with a concentration of 2% HSA (volume ratio of HSA/HBSS at 20 mL/L) at a density calculated to yield a final concentration of $(1\sim2)\times10^6$ cells per mL in each tube. The results had been shown that the yield samples and viability of PBNs was all more than ninety-five percent (Oh *et al*., 2008; Han and Wang, 2014).

Detection of purity and activity about PBNs

A few fresh isolated and purified re-suspended neutrophils were observed by Wright's staining under the optical microscope at high magnification and oil immersion lens. A total of one hundred neutrophils were randomly counted and their activity was subsequently calculated. Neutrophils activity = the number of neutrophils/100×100%. The freshly prepared 0.4% trypan blue was fully mixed with the neutrophils suspension at the volume ratio of 1:9, and the number of living cells and dead cells were all measured under microscope in less than 3 min. The percentage of living cells (%) = total number of living cells/(living cells + dead cells)×100%. Trypan blue staining results showed over 95% of the cells activity and purity were all viable (Wang *et al*., 2015).

Isolation of total RNA and establishment of reverse transcription system

The total RNA in PBNs of the patients and health controls were extracted with Trizol reagent, which were all reversed transcription into cDNA under the help of random primers. The reaction system was as follows: no RNase water 2 μL, 5×RT buffer 4μL, dNTP Mix (10m mol/L) 2μL, RNase inhibitor (20U/μL) 1μL, Oligo (dT)18 (0.5μg/μL) 1μL, total RNA 8μL, AMV RT (10U/μL) 2μL, total volume 20μL. After 37°C for 60 min and then 70°C for 10 min, and followed by instantaneous centrifuge for a few seconds. The cDNA was carefully packed and frozen in the refrigerator at -20°C.

Detection of CXCL8 mRNA

According to the sequence of human CXCL8 mRNA in GeneBank, the gene specific primers and probes were designed by software of Primer Express, and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was regarded as a internal reference. The primer sequences were designed based on Table I. Total RNA of PBNs was isolated by Trizol reagent and then reverse-transcribed to cDNA by oligo $(dT)_{18}$ primers. Positive and negative controls were all set up at the same time for comparison in each test. The amplification of cDNA by 1:5 dilution were carried out with 1.25 units of Taq DNA polymerase in 25 μL reaction mixture containing 10 pmol of specific primers, 2.5 mmol of $MgCl_2$, Ex-Taq 1.25 U, 2 mmoL/L of dNTPs, 1×PCR buffer (500 mmoL/L KCl, 100 mmoL Tris, 20 mg/mL gelatin, pH 8.3), 2×SYBRTM Green I and 2 μL of a standard substance or cDNA. The cDNA template was diluted in the proportion 1 in 5. The cycling conditions for GAPDA and CXCL8 were the same namely, preheated for 5 min at 94°C, followed by 30 cycles of heating at 94°C for 30 sec, 55°C for 40 sec, 72°C for 30 sec, and a final elongation for 5 min at 72°C, then cooling to 4°C until electrophoresis. The ratio of lgcDNA/lgGAPDH was regarded as the extreme level of CXCL8 mRNA.

Table I.- The primer sequences of CXCL8 and GAPDH.

Gene	Primers (5'→3')	Product size (bp)
CXCL8	F: CTT TGT CCA TTC CCA CTT CTG A	306
	R: TCC CTA ACG GTT GCC TTT GTA T	
GAPDH	F: ACC ACA GTC CAT GCC ATC AC	452
	R: TCC ACC ACC CTG TTG CTG TA	

Statistical analysis

The PCR products of GAPDH were regarded as an internal standard to determine quantitative amplification efficiency. The product was diluted to seven graded

concentrations (10^6, 10^5, 10^4, 10^3, 10^2, 10, and 0 copies/μL), which were used to plot the standard curve. The measurement data were expressed as mean±SD. Differences between means were assessed by analysis of variance and followed by Student's t test. The relationship was considered statistically significant when the P value was less than 0.05. The standard of correlation analysis was at $0 < |r| \leq 0.3$ for the weak correlation, $0.3 < |r| \leq 0.5$ for the low correlation, $0.5 < |r| \leq 0.8$ for the significantly relation and $0.8 < |r| \leq 1$ for the high relation.

RESULTS

Total RNA in PBNs

The different compositions in the fresh whole blood were respectively obtained after density gradient centrifugation with neutrophils isolation medium. The isolated and purified cell suspension was observed by routine smear and Wright's staining under the microscope at high magnification and oil immersion lens. The target cells were found with clear boundary, abundant cytoplasm with slightly pink and a lot of light purple tiny granules. The hypersegmented neutrophils were leukocytes in which there were generally three or more nuclear lobes, leaf and leaf between the filaments.

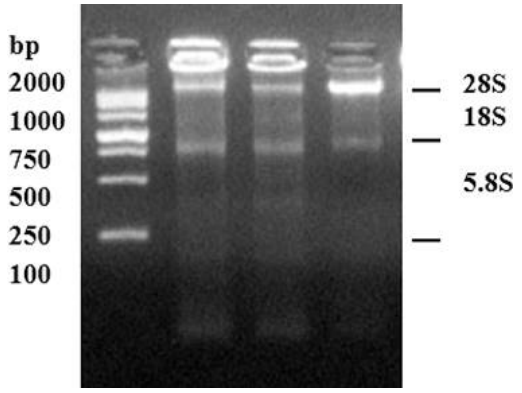

Fig. 1. The electrophorogram of total RNA from PBNs.

The concentration of PBNs was diluted to (1~2) ×10^6/mL with RPMI 1640 complete culture fluid and their total RNA was extracted by Trizol reagent. The total RNA 3 μL was mixed with bromophenol blue 1 μL, and taken in 1.2% TAE agarose gel (0.51 EB g/mL) electrophoresis for 25 min. Three fluorescent bands (28S, 18S, 5.8S) could be visible under the nucleic acid gel image analyzer and the density scanning camera was performed under the background of ultraviolet radiation. The density of 28S band was more than the twice of that of 18S band (Fig. 1). The results indicated that the total RNA was good, which could be satisfied with the requirements of the following experiments. The total RNA template was diluted in the proportion 1 in 250 and the value of OD260/OD280 was measured by ultraviolet spectrophotometry. When the value was ranged from 1.8 to 2, the total RNA purity was better and could be easily adequate for the following tests.

Expression of CXCL8 and its mRNA

The levels of CXCL8 and its mRNA in neutrophils of the thirty-seven patients with chronic renal failure were significantly higher. However, the difference was not significant (P>0.05). Further observation showed that the higher loads of CXCL8 mRNA were in the patients with infection than those in the patients without infection (P<0.05; P<0.01). CXCL8 and mRNA levels in peripheral blood of patients after hemodialysis for 12h were rapidly decreased, and no significant difference was found in the patients between with complicated infection and without complicated infection (P>0.05). Follow up observation in patients after treatment hemodialysis for third days, the levels of CXCL8 and its mRNA in peripheral blood have a certain degree of recovery, but there was no significant difference between the patients with complicated infection and without complicated infection (P>0.05). The details are shown in Figures 2 and 3.

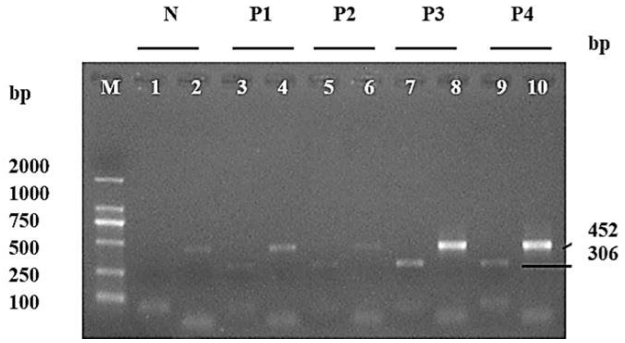

Fig. 2. Expression of CXCL8 mRNA in the PBNs of chronic renal failure. M, DL2000; 1 and 2, GAPDHC and CXCL8 of normal control; 3, 4, 5 and 6, GAPDH and CXCL8 of CRF without infection (patient 1 and patient 2); 7, 8, 9 and 10, GAPDH and CXCL8 of CRF with infection (patient 3 and patient 4).

Neutrophil count in patients with chronic renal failure

The count unit of neutrophils was defined as 109/L, absolute number of neutrophils was taken as the base of 10 for the logarithms, the logarithm values were regarded as the final level of numerical value. The counts of neutrophils in peripheral blood of chronic renal failure and normal controls were 9.6741±0.2537 and 9.6052±0.1204, respectively.

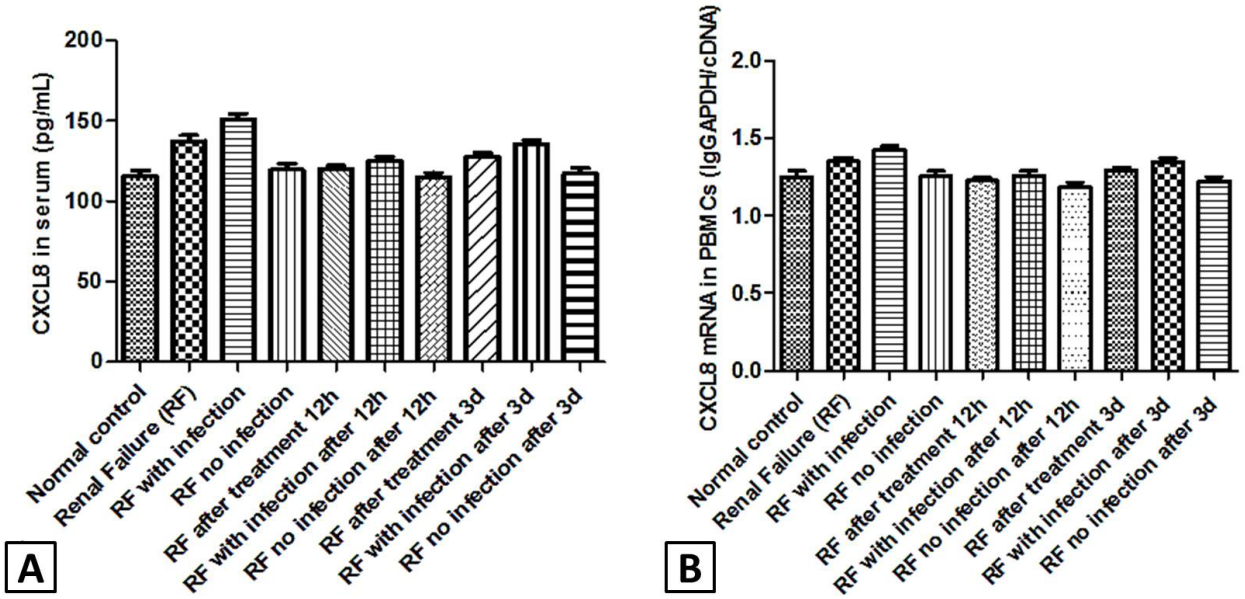

Fig. 3. levels of CXCL8 in serum (A) and CXCL8 mRNA in PBMCs (B) of chronic renal failure.

The few significant differences were found in two groups (P>0.05). The further comparative analysis found that the counts of neutrophils in the patients combined with infection and without infection were 9.8055±0.1399 and 9.5015±0.2690. The difference was significant (P<0.05) (Fig. 4).

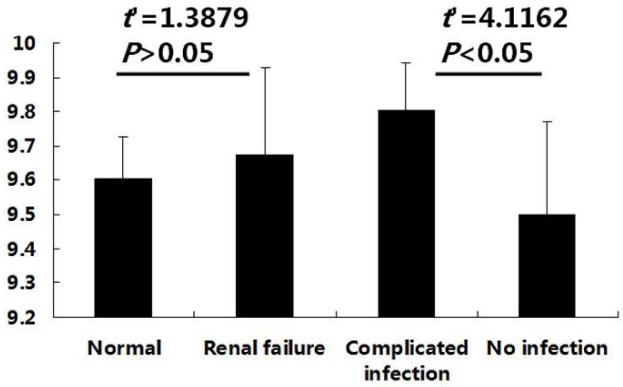

Fig. 4. The counts of neutrophil in peripheral blood of the patients with chronic renal failure.

Correlation of CXCL8 and its mRNA

As shown in Figures 5, 6 and 7, the overall correlation between the concentration of CXCL8 and expression of CXCL8 mRNA was lower in the peripheral blood of patients with chronic renal failure (r=0.4968). However, further comparative analysis showed that the content of CXCL8 was associated with its mRNA in the chronic renal failure patients complicated with infection (r=0.5707), and the poor correlation was identified in the patients without infection (r=0.02329). The correlation coefficient of CXCL8 and its mRNA in the peripheral blood of patients with chronic renal failure was significantly decreased after hemodialysis treatment for 12 h (r=0.3043). It was worth noting that the correlation coefficient between CXCL8 and its mRNA was more significant in the cases combined with infection (r=0.4226). The interval treatment of hemodialysis to the third days, the correlation coefficient of CXCL8 and its mRNA in patients with chronic renal failure complicated with infection was significantly increased again (r=0.4393).

DISCUSSION

CXCL8 also known as IL-8, or neutrophil factor, is a small molecule glycoprotein with a variety of biological activities of the molecular weight of about 8~10KD, is an important member of the ELR+ subfamily. It is secreted mainly from peripheral blood lymphocytes, monocytes, neutrophils and others, and has specific chemotaxis and activation to neutrophils (Meniailo *et al*., 2018), and plays an important role in the local and systemic response of anti-infection induced by neutrophils infiltration. In recent years, the role of neutrophils in the nonspecific immune response has been more and more attention in the world (Tecchio and Cassatella, 2016). In the pathological process of chronic renal failure, the renal ischemic damage was usually accompanied and can further induce glomerular

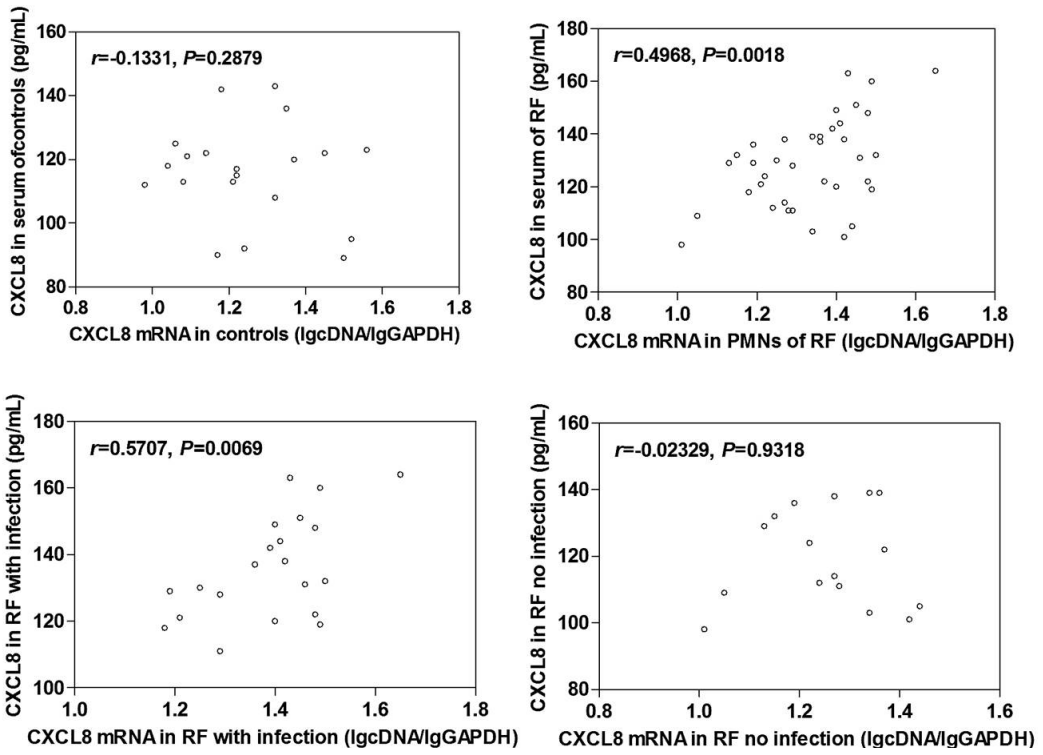

Fig. 5. Relationship of CXCL8 in serum and its mRNA in PBNs in patients of chronic renal failure (before hemodialysis).

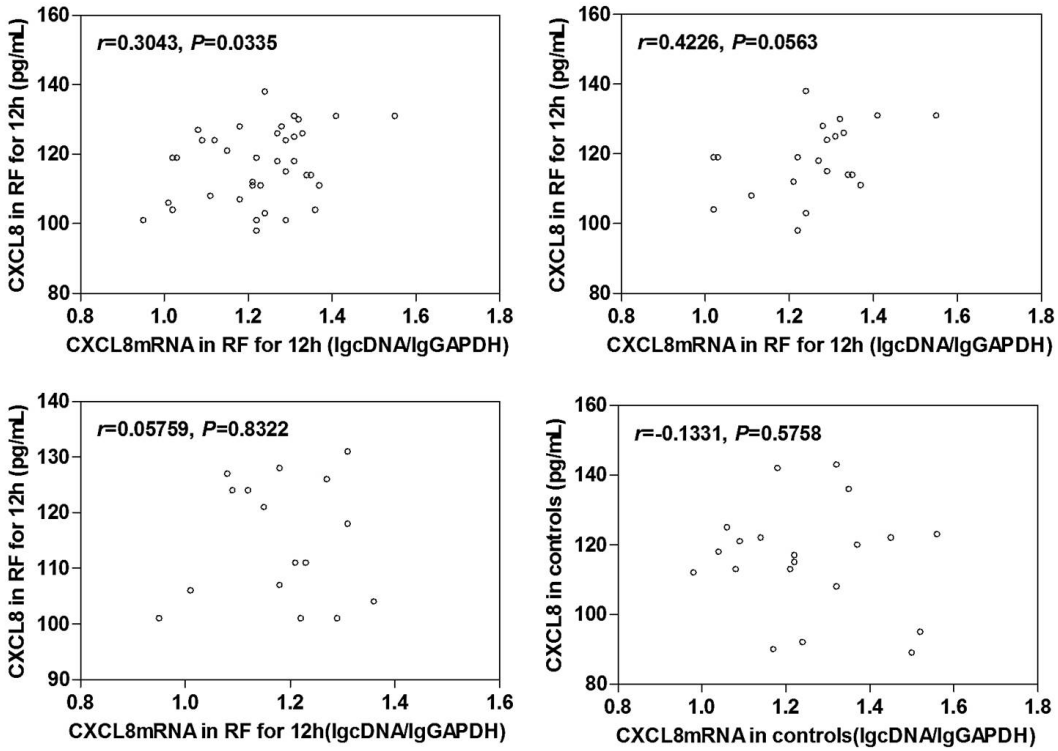

Fig. 6. Relationship of CXCL8 in serum and its mRNA in PBNs in patients of chronic renal failure (after hemodialysis for 12h).

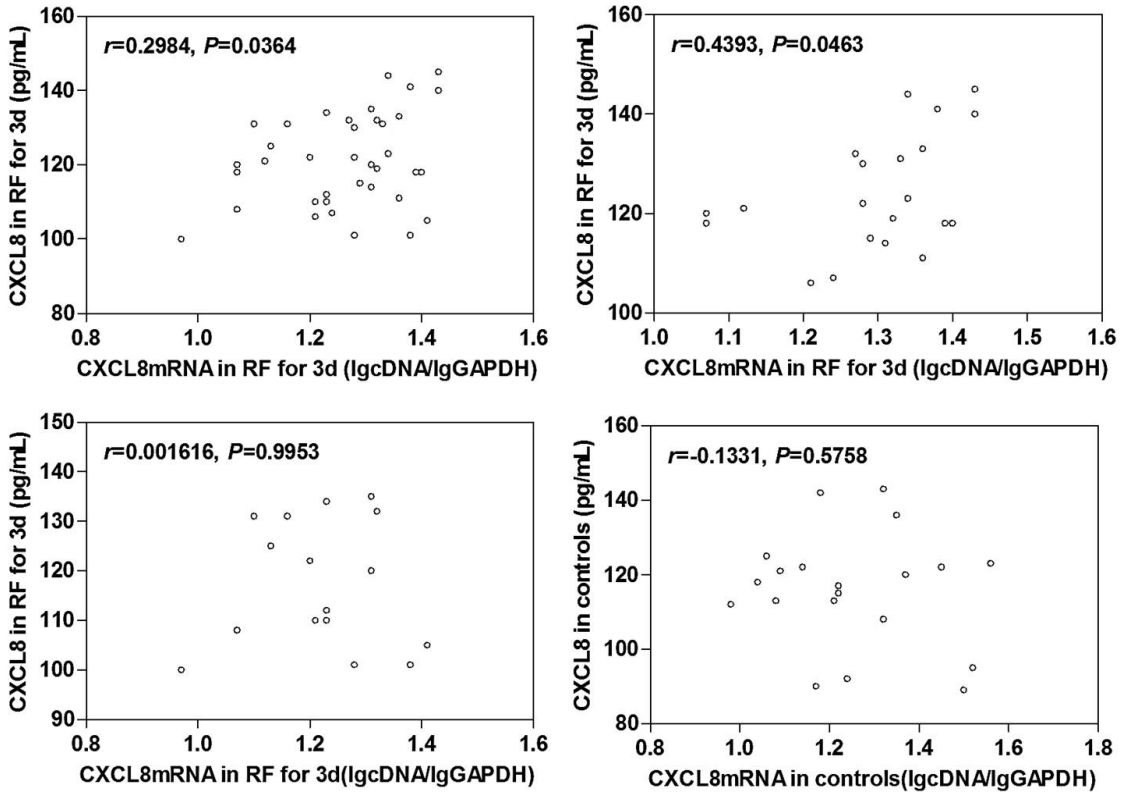

Fig. 7. Relationship of CXCL8 in serum and its mRNA in PBNs in patients of chronic renal failure (after hemodialysis for 3d).

capillary loops collapse, so that a large number of glomerular extracellular matrix (ECM) accumulation and glomerular mesangial cell proliferation and base membrane thickness could be observed (Ruiz-Torres *et al*, 2005), and the related vascular inflammation was also induced. Neutrophils, a group of non-specific immune cells, can accumulate in the organization and selectively chemotaxis to the inflammatory sites, the lysosomal enzymes and superoxides from neutrophils play the role of phagocytosis and damage effects against the apoptotic cells and the pathogen, and also induce the permeability of vascular endothelial cells, and then participate in the local tissue injury and repair (Jones *et al*., 2016; Feng *et al*, 2014).

The data in our studies had been shown that the levels of CXCL8 and its mRNA in the patients with renal failure and were all higher than those of the normal controls, but the difference was not significant (t=2.8468, P=0.062; t=1.953, P=0.0559). It suggested that a certain degree of microinflammatory response might be existed in chronic renal failure. Fortunately, the inflammation was not active *in vivo* (Memoli *et al*., 2010). Further comparative analysis had been found that the levels of CXCL8 and its mRNA were all higher in the chronic renal failure complicated with infection than those without infection (t=3.2901, P=0.023; t=3.3701, P=0.0018). The results clearly showed that a significant local or systemic inflammatory response had been found in the chronic renal failure associated with infection. The reason for this phenomenon might be: First, kidney itself is insufficient to remove the toxic metabolic waste and CXCL8 and other inflammatory chemokines in circulation (Okhunov *et al*., 2016). Second, multiple cytokines, such as IL-6, IL-8, TNF-α, could be released from the target cells in the chronic renal failure complicated with local infection in kidney or systemic infection, and were known to induce adhesion and migration of neutrophils, lymphocytes and others to the renal interstitial aggregation. The inflammatory reaction was triggered by activated oxygen and protease, and further induced injury of the renal tubular epithelial cells (Murugan *et al*., 2014). Third, inflammation was caused by micro dose of the endotoxin and bacterial contamination in reverse osmosis water and dialysis fluids in the process of hemodialysis. Dialysis membrane could directly filter resistance bacteria pollution, but some soluble metabolites might still penetrate through the dialysis membrane from the catheter system into the blood. Activation of neutrophils, monocytes macrophages and other immunocytes secreted

a large number of pro-inflammatory factors, so that the level of CXCL8 in peripheral blood would be increased accordingly (Kashiwagi *et al*., 2014; Koda *et al*., 2017). In addition, according to the analytics on big clinical data of United States Renal Data System (USRDS), the inflammatory response in patients with chronic renal failure was obviously related to the genetic background of the individual (Cheng *et al*., 2013).

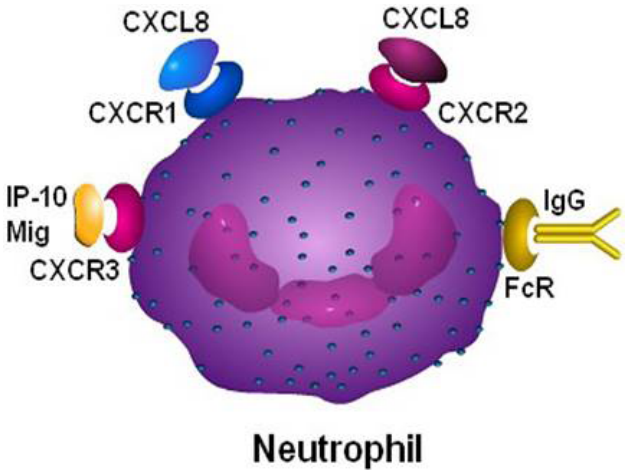

Fig. 8. Neutrophil and its major receptors. Neutrophil can express CXCR1, CXCR2, FcR and other a variety of chemokine receptors, which may combine with the corresponding ligands, and further participate in local and systemic inflammatory damage and repair. IP-10, interferon-inducible protein-10; Mig, monokine induced by the interferon-gamma.

Neutrophils derived from bone marrow hematopoietic stem cells, which undergoes multiple developmental stages, such as myeloblast, premyelocyte, myelocyte, metagranulocyte, band neutrophils, and eventually develop into mature neutrophils. Neutrophils are the main component of leukocytes in the human circulation, which percentage accounting for 60%~70%, and are the first non-specific immunocytes to arrive at the site of infection through the pathway of transendothelial migration. Mature neutrophils in the circulation can be rich in various receptors, such as CXCR1, CXCR2, FcγR (Fc gamma receptor) and other receptors (Russo *et al*., 2014; Sun *et al*., 2016) (Fig. 8). Combination of IL-8 and CXCR1 or CXCR2 at high-affinity can activate target cells (such as human polymorphonuclear leukocytes) and further activate the downstream tyrosine kinase cascade signal transduction pathway (Futosi and Mócsai, 2016), produce a host of chemotactic small molecules, and recruit more neutrophils quickly migrating through the vascular endothelial cell to facilitate their participation in local or systemic tissue injury and repair (de Oliveira *et al*., 2016; El-Benna *et al*., 2016). The results of this study showed that the number of neutrophils in peripheral blood of the patients with chronic renal failure was not significantly increased or decreased. It was noted that the number of neutrophils in patients combined with infection was significantly increased. It had been confirmed that the activation of neutrophils, monocytes, macrophages under repeated stimulation of the multiple inflammatory cytokines could be abundantly expressed CXCR1, CXCR2. CXCL8 could induce the chemotaxis migration of target cells through interaction with the specific receptor CXCR1 and CXCR2 to involve in the inflammatory response (Gupta and Kaplan, 2016; Wang *et al*., 2017).

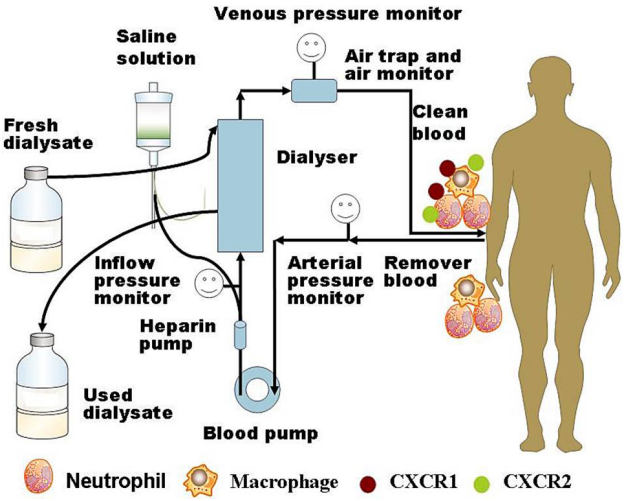

Fig. 9. The main process of hemodialysis and its activation on neutrophils and mononuclear macrophages. When multiple toxic metabolites in the blood stream were filtered by hemodialyzer, the activity of neutrophils and mononuclear macrophages in the circulation gradually increased.

Blood purification system includes hemodialysis (HD), hemofiltration (HF) and hemoperfusion (HP) and other modes, their basic principles of hemodialysis systems are based on diffusion, ultrafiltration, adsorption and others, and further removal of toxins and metabolic waste from the blood. It can keep a balance of electrolyte, acid and alkali in body, and finally maintains the stability of the internal environment close to normal level in the organism (Fig. 9) (Fonseca *et al*., 2016). It can reduce the autoimmune response caused by excessive stimulation of toxic metabolites on neutrophils, instead of the partial excretory function of the normal kidney so as to sustain the life of the patients. In this paper, the dynamic observation results had shown that hemodialysis could effectively

eliminate toxic metabolites and remove the initial factor induced inflammatory responses generated in the patients, significantly reduced the accumulation of toxic products on the stimulation to neutrophils, so that the secretion ability of CXCL8 apparently decreased and closed to the normal level. The regularity with dynamic change is more obvious in the patients with infection (Safa *et al*., 2014). When hemodialysis was in intermittent treatment for the third day, because the filter function of kidney in the patients was obviously decreased, the original remnants of the inflammatory factors, along with the accumulation of subsequent metabolic waste might invade into the blood circulation again. These compounds co-stimulated the neutrophils, monocytes and macrophages to activate and secrete a variety of cytokines, chemokines, so that the micro inflammatory state must continue to be repeated or poor (Banerjee *et al*., 2017; Taraz *et al*., 2015). Therefore, the intermittent and continuous hemodialysis therapy must be provided to the patients with chronic renal failure or loss of renal function, so as to inhibit a variety of metabolic waste and inflammatory factor in a very low level, and to keep the stability of the micro environment in the body, not to endanger the lives of the patients. The results of Pereira *et al*. (2010) based on 34 chronic kidney disease patients treated with HD and recombinant human erythropoietin treatment showed that the level of CXCR1 on surface of neutrophils in the patients was decreased before hemodialysis, and the levels of CXCR1, CD14 and CD11b on the surface of neutrophils were significantly increased after hemodialysis. However, comparing with the controls, only the HLA-DR level on the surface of monocytes was significantly increased. It suggested that hemodialysis could regulate the activation of neutrophils and monocytes, and the activated neutrophils and monocytes were actively involved in the inflammatory response, which was conducive to the repair of inflammatory injury (Wang *et al*., 2018).

The messenger RNA of CXCL8 plays an important role in the regulation of CXCL8 secretion by host cells (Lin *et al*., 2016). Our clinical trials corroborated that the expression of CXCL8 mRNA and concentration of CXCL8 showed only low correlation in peripheral blood of the patients with chronic renal failure (r=0.4968, P=0.0018). But the comparison analysis in different group easily found that a moderate degree of relevance to CXCL8 and its mRNA could be set up in the CRF patients complicated with infection (r=0.5707, P=0.0069). In contrast to this, the weak correlation of CXCL8 and its mRNA was found in the CRF patients without complicated infection (r=0.02329, P=0.9318). It prompted that CXCL8 mRNA was indeed involved in the regulation of the secretion of CXCL8 in host cells, and was closely related to the complicated infection. The different infection factors actively promoted the transcription of the messenger mRNA about CXCL8 so that the free level of CXCL8 in serum was significantly increased. The results of our clinical dynamic observation demonstrated that the correlation coefficient of CXCL8 and its mRNA was decreased after treatment of hemodialysis for 12 h (r=0.3043, P=0.0335), and the similar results were more significant in the patients complicated with infection (r=0.4226, P=0.0563), and approached the normal level at 12 h in hemodialysis. The clinical trials illustrated that hemodialysis could directly clean a variety of adverse stimuli effects come from metabolic products in blood circulation of host and remove the factors in the initial stage of inflammation so as to maintain the relative stability of the micro environment in vivo, and also can promote the repair of renal microvascular injury (Almeida *et al*., 2015; Mitsides *et al*., 2017), and successfully as well as improve their quality of life. When the patients were at the end of the blood dialysis intermittent treatment, also namely on the 3rd day after hemodialysis, the toxic metabolites and micro inflammatory factors would be re-accumulated in the patients with renal dysfunction. The new metabolic waste was far beyond the capacity of the kidney and repeatedly stimulated neutrophils, monocytes and macrophages. The transcription activity of the messenger mRNA of CXCL8 was increased in activated target cells, the correlation coefficient of CXCL8 and its mRNA re-increased in the chronic renal failure patients complicated with infection (r=0.4393, P=0.0463). Therefore, hemodialysis must be promptly provided for these patients in order to filter out new metabolic waste, to keep the various metabolites in blood circulation at a low level, and finally improve the quality of life and prolong the survival time of the patients.

In summary, the CRF patients are usually accompanied by obvious micro inflammatory reaction (Viaene *et al*., 2016; Mitsides *et al*., 2017), which caused by high level of CXCL8 and its mRNA. The patients with complicated infection were more significant. A large number of metabolic waste and a variety of inflammatory cytokines can induce the activation and proliferation of neutrophils. Activated neutrophils can express a large number of CXCR1 and CXCR2, and specifically bind to its ligand CXCL8, so that many target cells may be attracted and migrated to the sites of inflammation, and participate in the micro inflammatory reaction of chronic renal failure (Shahida *et al.,* 2017). Hemodialysis is low at costs, and also simple and safety on operation. It can effectively remove multiple initial factors evoked inflammatory reaction in blood circulation, so as to maintain a relatively stable in microenvironment in vivo. In order to consolidate the curative effects in clinical trials, the continuous and intermittent hemodialysis should be usually provided to

the patients with chronic renal failure.

CONCLUSIONS

The over high levels of CXCL8 and its mRNA are closely correlated with the merger of infection, and can be filtered out and by hemodialysis in the short term. The intermittent hemodialysis can effectively improve the micro inflammatory response in the patients with chronic renal failure.

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Statement of conflict of interest

All authors declare that there is no conflict of interests regarding the publication of the manuscript.

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