CRP, IL-2 and TNF-α level in patients with uremia receiving hemodialysis

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Abstract. Uremia is a serious threat to health. Infection associated with inflammation frequently occurs in patients with uremia during hemodialysis. This study aimed to investigate the association between serum inflammatory factors and uremia in patients prior to and following hemodialysis. Patients with uremia (n=200) receiving continuous high throughput hemodialysis that had hospital-acquired infection were enrolled between August 2013 and August 2015. Additionally, 200 cases of healthy volunteers were selected as the control. Reverse transcription-polymerase chain reaction, ELISA and western blotting were performed to determine serum C-reactive protein (CRP), interleukin 2 (IL-2), and tumor necrosis factorα levels (TNF-α) prior to hemodialysis and 8 months after hemodialysis to explore the association of CRP, IL-2 and TNF-α with uremia. CRP, IL-2 and TNF-α levels were lower at 8 months after hemodialysis than before, and the difference was statistically significant. CRP, IL-2 and TNF-α levels in uremia patients at 8 months after hemodialysis were similar with that in the normal control. CRP expression in patients with uremia was positively correlated with IL-2 and TNF-α expression. Patients with uremia with hospital-acquired infection receiving continuous high throughput blood purification presented increased levels of inflammatory factor in the serum. In conclusion, uremia patients receiving maintenance hemodialysis with hospital-acquired infection had increased serum inflammatory factors and high throughput hemodialysis significantly decreased CRP, IL-2 and TNF-α levels in the serum, suggesting that high throughput hemodialysis may be beneficial for the prevention of the infections in uremia patients.

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Introduction

The incidence of uremia (urea in the blood) has exhibited an increasing trend following increasing in living standards and lifestyle changes. Uremia is often accompanied by high mortality and complications that severely threaten health (1-3). Hemodialysis and purification is one of the main methods for treatment of uremia. Hemodialysis uses dialysis membrane to exchange or eliminate small molecules from patient blood and dialysate, to return the blood components of normal level (4,5). With the improvement of science and healthcare, hemodialysis and purification technology has significantly reduced the mortality rate of uremia and improved patient quality of life. However, hemodialysis and purification also cause infection, which seriously restricts uremia treatment (6-8). Therefore, clarification of the molecular mechanism that are induced in patients with uremia receiving hemodialysis, and taking effective counter measures, is urgently required (9).

Previous studies reported that abnormal protein factors in the serum of patients with uremia may be associated with cardiovascular complications, malnutrition and high mortality (10-12). Furthermore, it was revealed that inflammation was closely associated with uremia occurrence, development and prognosis (13). Inflammatory status in uremic patient was improved after hemodialysis, suggesting that may be associated with serum inflammatory factors level (14).

Hemodialysis and purification are the important methods for the treatment of end-stage renal disease. Maintenance hemodialysis (MHD) increases the survival of patients with uremia (15-17). However, the incidence of complications gradually increased following hemodialysis and purification, suggesting that inflammation may have an important role in occurrence and development of complications following MHD (18-20). Inflammation is often accompanied by abnormal organ functions and changes in hepatic protein levels. Specifically, C-reactive protein (CRP), interleukin-2 (IL-2) (21) and tumor necrosis factor-α (TNF-α) (21-24) are important serum markers of inflammation.

Thus, the current study detected CRP, IL-2 and TNF-α levels in patients with uremia receiving hemodialysis, aiming to improve the effectiveness of hemodialysis and provide valuable information for to reduce inflammation.

Materials and methods

Experimental subjects and grouping. Patients with uremia were enrolled according to the following inclusion (25) and exclusion (26) criteria: i) Hemodialysis for 6 months or longer; ii) no blood transfusion or hemorrhage in 6 months; iii) no infection, trauma, surgery or tumor in previous 2 months; iv) no other types of blood system disease. The causes of abnormal renal function and the number of cases were as follows: 2 refluxnephropathy, 4 gout kidney disease, 4 interstitial nephritis and chronic nephropyelitis, 10 polycystic kidney, 72 chronic glomerulonephritis, four hypertensive renal arteriolar sclerosis, 20 diabetic nephropathy, the remaining patients had ≥one of the above complications. Patients with uremia (n=200) receiving continuous high throughput blood purification along with the hospital-acquired infection, as determined by routine tests in the hospital, in the First Affiliated Hospital of Henan University of Science and Technology (Luoyang, China) from August 2013 and August 2015 were enrolled in the study. Additionally, 200 healthy volunteers were selected as control group. The basic patient information is listed in Table I. There were 154 male and 46 female patients with uremia, with mean age 47.4±12.3 (18-70) years old. The mean age of the control group (healthy volunteers) was 48.3±13.2 (18-70) years old. Experimental protocols were submitted to and approved by the ethics committee of the First Affiliated Hospital of Henan University of Science and Technology. Written informed consent was provided.

Therapeutic method. In 200 patients receiving hemodialysis, 58 patients received hemodialysis six times per week (24 h). Regular dialysis was maintained for 6 months or longer, and Kt/V >1.2. The treatment was in accordance with the clinical practice guidelines of renal anemia (27). Human erythropoietin was injected at 8,000-12,000 IU per week according to the hemoglobin level. Fresenius (F8) and Baxter dialyzators were used for hemodialysis. Bicarbonate was the dialysate, flow rate was 600 ml/min and the blood flow was 400-600 ml/min.

Blood sample collection. Blood samples were collected according to the regular method (28). Fasting venous blood was extracted from 200 patients with uremia for detection of serum inflammatory factors. Following hemodialysis and purification, 5 ml blood was collected and incubated at 37˚C for 30 min. Then the supernatant was collected after centrifuged at 1,500 x g for 8 min at 4˚C. Part of the serum was used for ELISA detection, and the other was used for reverse transcription (RT)-polymerase chain reaction (PCR). All the procedures were completed within 60 min. Blood was collected on the day prior to hemodialysis in the F group and 8 months post-hemodialysis in the R group.

Reagents and primers. IL-2 (cat no. 04-1584), TNF-α (cat no. T8300), CRP (cat no. C1688) and β-actin (cat no. A2228) antibodies were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). IL-2, TNF-α, CRP primers were as follows: IL-2, forward 5'-TGTCCAGATGTAAGTAATAAAACAGAACA-3' and reverse 5'-CAGAATGTAAGTAATGTCAAATCAGAACA-3'; TNF-α, forward 5'-AACACCTCTTTACAGTGACCAATGCCCCA3' and reverse 5'ACAGTGACTAATTTCCAACACCTGCCCCA3'; CRP, forward 5'-TTACAGTGACCAACACCTCTAATGCCCCA-3' and reverse 5'CGTGAAACACCTACACTAATTCTGCCCCA3'; β-actin, forward 5'-CACCAACTGGGACGACAT-3' and reverse 5'-ACAGCCTGGATAGCAACG-3'.

The primers were designed and synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China).

Table I. Basic patient information.

Group	Number of cases	Male (%)	Mean age
Test	200	77	47.4±12.3
Control	200	77	48.3±13.2

ELISA. ELISA was performed to determine the level of serum inflammatory factors IL-2 (cat no. ab46054; Abcam, Cambridge, UK), TNF-α (cat no. ab46087; Abcam), CRP (cat no. ab99995; Abcam) in serum (18). The antibody was diluted to 5 μg/ml in coating buffer and added to each well at 0.1 ml. After water bathed at 37˚C for 2 h, the plate was washed in washing buffer six times. Subsequently, 0.1 ml sample was added at 37˚C for 60 min, then the plate was washed in washing buffer for six times and the absorbance was measured at 490 nm with a microplate reader.

RT-PCR. RT-PCR was performed to determine the mRNA levels of inflammatory factors using standard procedures (17). Total RNA was isolated using TRIzol reagent followed by reversely transcription into cDNA using a Universal RT-PCR kit (Beijing Dingguo Biotechnology Co., Ltd., Beijing, China). Briefly, the mixture from Universal RT-PCR kit was added to the isolated RNA and then placed at 25˚C for 10 min followed by incubation at 42˚C for 1 h. After that, the cDNA was denatured at 95˚C and placed on ice for PCR analysis. The reaction system contained 2 μl cDNA solution, 2.5 μl 10X PCR Buffer, 2.5 μl dNTP mixture (2 mM), 0.5 μl primer 1 (10 μM), 0.5 μl primer 2 (10 μM), 0.5 μl Taq DNA Polymerase, 2.5 μl $MgCl_2$ (25 mM) (all from Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 14 μl H_2O. The reaction conditions were as follows: 94˚C for 7 min, followed by 30 cycles of 94˚C for 30 sec, 50˚C for 60 sec and 72˚C for 30 sec, and a final step of 72˚C for 5 min. Image J software version 1.4 (National Institutes of Health, Bethesda, MD, USA) was used to semi-quantify the RT-PCR bands. The relative expression of inflammatory factor genes was calculated by the ratio of the band grey of the inflammatory factors and actin.

Western blot analysis. Western blot was performed to detect inflammatory factor protein level in blood samples (16). Briefly, total protein was extracted using radioimminoprecipitation lysis buffer (Thermo Fisher Scientific, Inc.) and was quantified using a bicinchoninic acid assay. A total of 40 μg per lane protein was loaded into 10% SDS-PAGE, followed by transferring to polyvinylidene difluoride membrane and then blocked with 5% defatted milk powder for 2 h at room temperature. Primary antibodies against IL-2 (cat no: EPR2780; Abcam), TNF-α cat no: ab9635; Abcam, CRP (cat no. ab50861; Abcam) or β-actin (cat no. ab8227; Abcam) (1:1,000 or 1:2,000

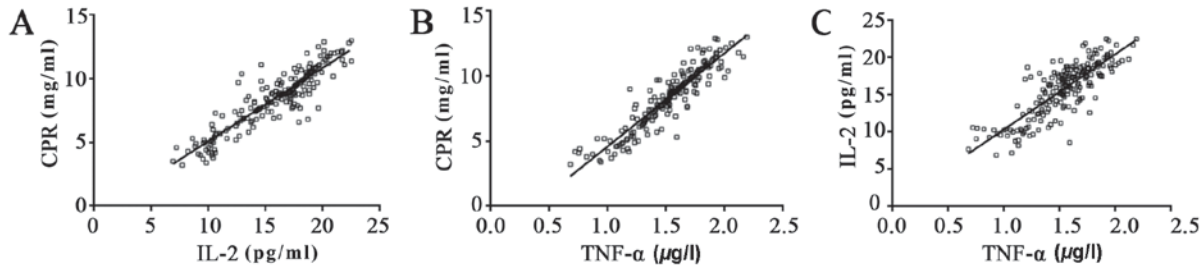

Figure 1. Correlation analysis among uremia patients prior to hemodialysis. (A) IL-2 vs. CRP; (B) TNF-α vs. CRP; (C) TNF-α vs. IL-2 (n=200). CRP, C-reactive protein; IL-2, interleukin-2; TNF-α, tumor necrosis factor-α.

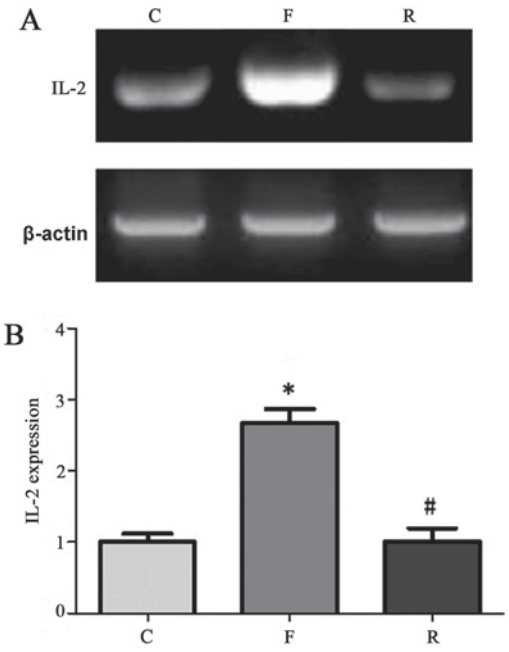

Figure 2. IL-2 mRNA level. (A) IL-2 RT-PCR results. (B) RT-PCR semi-quantification (n=90). *P<0.05 vs. C. #P<0.05 vs. F. RT-PCR, reverse transcription-polymerase chain reaction; IL-2, interleukin-2; C, normal control; F, prior to hemodialysis; R, 8 months post-hemodialysis.

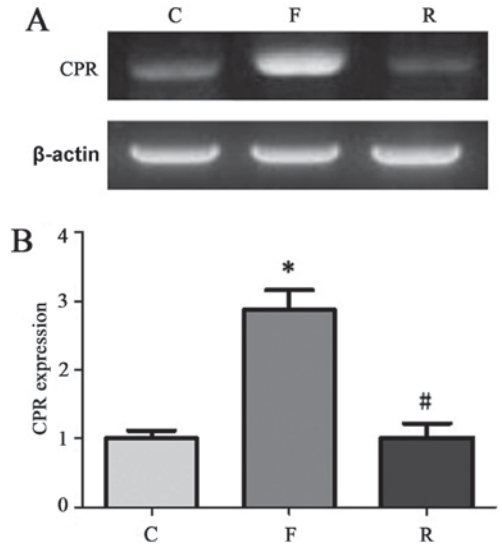

Figure 3. CRP mRNA level. (A) CRP RT-PCR results. (B) RT-PCR semi-quantification (n=90). *P<0.05 vs. C. #P<0.05 vs. F. RT-PCR, reverse transcription-polymerase chain reaction; CRP, C-reactive protein; C, normal control; F, prior to hemodialysis; R, 8 months post-hemodialysis.

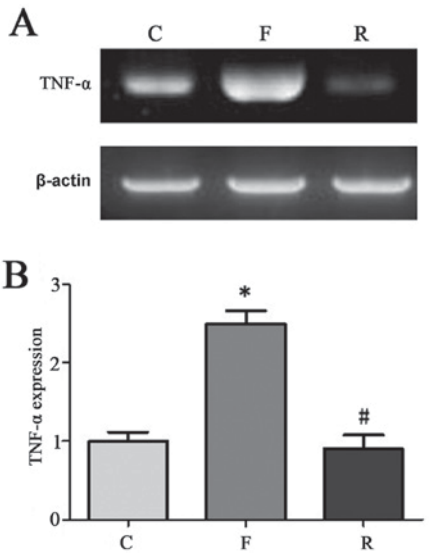

Figure 4. TNF-α mRNA level. (A) TNF-α RT-PCR results. (B) RT-PCR semi-quantification (n=90). *P<0.05 vs. C. #P<0.05 vs. F. RT-PCR, reverse transcription-polymerase chain reaction; TNF-α, tumor necrosis factor-α; C, normal control; F, prior to hemodialysis; R, 8 months post-hemodialysis.

dilutions) were added overnight at 4˚C. After PBST (0.1% Tween-20) washing, goat anti-rabbit (cat no. ab97051; Abcam) or rabbit anti-mouse secondary antibodies (cat no. ab6728; Abcam) (1:5,000) were then added and incubated in the dark at room temperature for 30 min. Enhanced Chemiluminescence (Thermo Fisher Scientific, Inc.) reagent was then used to develop the membrane for 1 min, followed by X-ray exposure and observation. Image J software version 1.4 was used to analyze western blot bands. Inflammatory factors protein relative expression was calculated by the ratio of the band grey of inflammatory factors and actin.

Statistical analysis. SPSS13.0 was used for statistical analysis. All data was presented as the mean ± standard deviation. One-way analysis of variance was performed to assess the statistical significance among multiple treatment groups followed by Least Significant Difference test as the post hoc test. Pearson correlation was conducted for the correlation analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

Correlation analysis of IL-2, TNF-α and CRP. In samples taken from 200 uremia patients prior to dialysis, ELISA data

Table II. Serum IL-2, TNF-α, and CRP protein expression comparison.

Group	Cases	IL-2 (pg/ml)	TNF-α (μg/l)	CRP (mg/l)
Control	200	6.2±1.9	0.64±0.32	2.8±0.8
Pre-dialysis	200	15.7±2.8[a]	1.53±0.56[a]	8.4±1.6[a]
Post-dialysis (8 months)	200	6.9±2.2[b]	0.69±0.41[b]	3.1±1.1[b]

IL-2, interleukin-2; TNF-α, tumor necrosis factor-α; CRP, C-reactive protein. [a]P<0.01 vs. control; [b]P<0.01 vs. pre-dialysis.

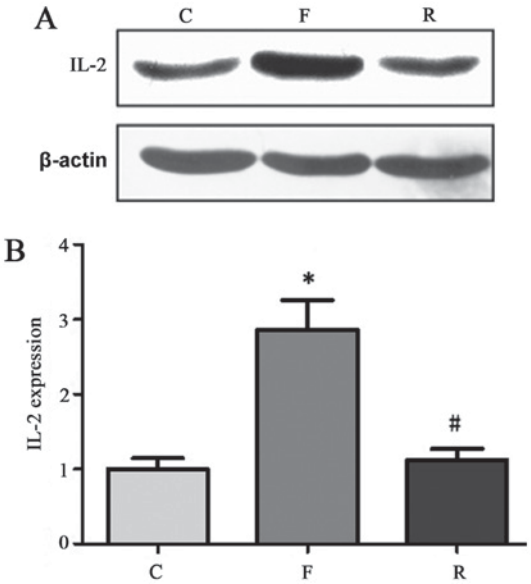

Figure 5. IL-2 protein level. (A) IL-2 western blot results. (B) Western blot quantification (n=30). *P<0.05 vs. C. #P<0.05 vs. F. IL-2, interleukin-2; C, normal control; F, prior to hemodialysis; R, 8 months post-hemodialysis.

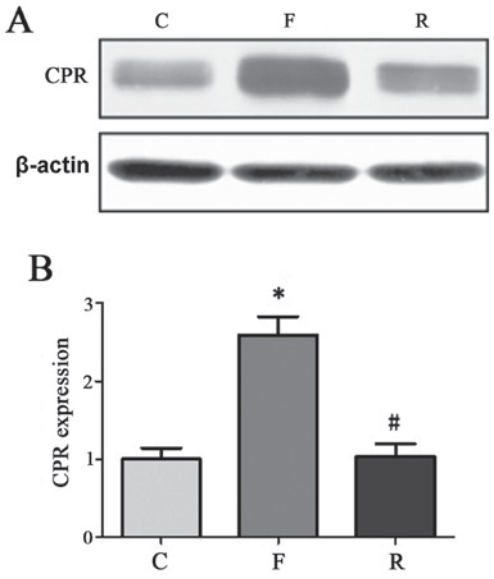

Figure 7. CRP protein level. (A) CRP western blot results. (B) Western blot quantification (n=30). *P<0.05 vs. C. #P<0.05 vs. F. CRP, C-reactive protein; C, normal control; F, prior to hemodialysis; R, 8 months post-hemodialysis.

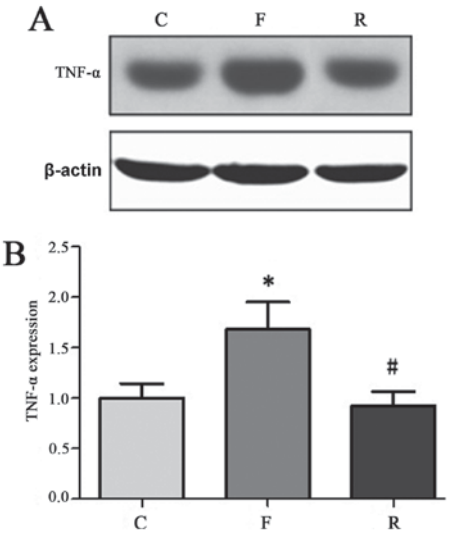

Figure 6. TNF-α protein level. (A) TNF-α western blot results. (B) Western blot quantification (n=30). R, uremia patients after receiving hemodialysis. *P<0.05 vs. C. #P<0.05 vs. F. TNF-α, tumor necrosis factor-α; C, normal control; F, prior to hemodialysis; R, 8 months post-hemodialysis.

indicated that the IL-2 level was positively correlated with CRP (r^2=0.8245; P<0.05), TNF-α was positively correlated with CRP (r^2=0.8513; P<0.05) and TNF-α was positively correlated with IL-2 (r^2=0.684; P<0.05), which indicated that IL-2, TNF-α and CRP may be useful as bio-markers in the diagnosis of uremia (Fig. 1).

Serum IL-2, TNF-α, and CRP mRNA expression. We randomly selected 90 samples among the 200 patients for RT-PCR detection. The results indicated that CRP, IL-2 and TNF-α levels were significantly lower at 8 months after hemodialysis compared to before treatment (P<0.05; Figs. 2-4). CRP, IL-2, and TNF-α levels in patients with uremia at 8 months after hemodialysis were similar with that in normal control.

Serum IL-2, TNF-α, and CRP protein expression. ELISA was conducted to analyze blood samples from 200 patients with uremia and controls. The results indicated that CRP, IL-2, and TNF-α levels were reduced at 8 months after hemodialysis compared with before treatment. CRP, IL-2, and TNF-α levels in uremia patients at 8 months after hemodialysis were similar to that in normal control samples (Table II). Western blot analysis validated this further. In 30 randomly-selected samples from the 200 patients CRP, IL-2 and TNF-α protein levels were significantly decreased at 8 months after hemodialysis compared with before treatment (P<0.05; Figs. 5-7).

Symptoms improvement comparison after therapy. After 8 months of hemodialysis, the patient's symptoms of uremia, sleep and appetite obviously improved. In addition, other symptoms, such as hypertension, peripheral neuropathy and renal bone disease were also improved to a certain extent with some even being recovered.

Adverse reaction. During the 8 months of hemodialysis, six cases exhibited perspiration and precordial discomfort at 30 min after hemodialysis treatment. For all these six cases, this may be the first time a new dialyzer replacement was performed. After administration with hypertonic glucose and dexamethasone, the symptoms of these six patients were effectively relieved. No other symptoms appeared during the following treatment period. Most patients showed good tolerability with no other adverse reactions. No patients opted out of the experiment over the whole treatment process.

Discussion

Uremia is a serious threat human health (1). Infection often occurs during the process of hemodialysis, which can aggravates patient conditions (3). It is necessary to investigate the levels serum inflammatory factors to improve the effect of hemodialysis and provide valuable information for developing anti-inflammatory treatments.

It was previous revealed that various metabolites are abnormally accumulated in the serum of patients with uremia (16,17). Hemodialysis and purification can alleviate the abnormal accumulation of metabolites maintain normal concentrations in the blood (18), suggesting that abnormal accumulation of serum metabolites may be important factors involved in uremia and dialysis-associated complications. The results of the current study suggested that CRP, IL-2 and TNF-α levels were lower at 8 months after hemodialysis than before treatment, and the difference was statistically significant. Additionally, CRP, IL-2 and TNF-α levels in patients with uremia at 8 months after hemodialysis were similar to that in normal control samples. CRP expression in patients with uremia was positively correlated with IL-2 and TNF-α expression levels.

There were two important findings in the current study. Firstly, patients with uremia that received MHD and purification combined with hospital infection exhibited increased levels of inflammatory factors in their serum compared with the control group. Additionally, high throughput dialysis and purification significantly reduced serum CRP, IL-2, and TNF-α levels.

A previous study confirmed that uremia is often accompanied by a chronic inflammatory response (12). We speculated that inflammatory cytokines level may change. The results suggested that serum CRP, IL-2 and TNF-α levels were elevated prior to hemodialysis compared with the control group, which was in accordance with the previous research (16). The potential mechanism is that decreased immune function together with peritoneal access or vasodilation in patients with uremia leads to bacterial contamination and a series of complex responses. Subsequently, the complement system is quickly activated, resulting in an inflammatory response.

The current study has various limitations. The number of patients enrolled in the study, was limited because of time and other factors, and it may affect the reliability of the results. Additionally, certain studies have reported that inflammation in the dialysis patients is mainly induced by the type of dialysis membrane (29,30); the association between the different dialysis membranes and the inflammation factors was not investigated in this study. Furthermore, the present study did not investigate the chronic inflammatory state for enrolled patients. These require attention in future studies.

In conclusion, uremia patients receiving MHD with hospital-acquired infection had increased serum inflammatory factors. Following high throughput hemodialysis, the levels of CRP, IL-2 and TNF-α were significantly decreased in patient serum, suggesting high throughput hemodialysis may be beneficial for prevention of the infections in uremia patients.

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