European Review for Medical and Pharmacological Sciences 2018; 22: 4598-4603

Preliminary study of Bim on the early diagnosis and prognosis of the elderly uremia with gastrointestinal nutrition combined with dialysis

M. YANG[1], Y.-H. BAI[1], J.-S. WANG[2], H.-Y. JIANG[1], S.-L. HU[1]

[1]Department of Nephrology, and [2]Department of Urology; The Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, China

Abstract. – OBJECTIVE: Elderly uremia frequently refers to the end stage of various chronic kidney diseases that threats the patients' health seriously. Enteral nutrition can reduce complications, while the molecular mechanism is still unclear. Mitochondrial protein Bim plays an essential role in regulating inflammation, restraining oxidative stress, and maintaining the balance of the mitochondrial membrane potential and energy production. This study aims to investigate the effect of Bim on the early diagnosis and prognosis of the elderly uremia with gastrointestinal nutrition combined with dialysis.

PATIENTS AND METHODS: Elderly patients with uremia in our hospital were selected and divided into parenteral nutrition group, enteral nutrition group, and regular treatment group. Healthy volunteers were chosen as the control group. Blood oxygen free radicals were tested by flow cytometry. Blood immune function parameter C-reactive protein and IL-6 levels were determined by Western blot. Bim expression in blood was evaluated by RT-PCR and Western blot. Correlation analysis was performed between Bim level and the prognosis of elderly patients with uremia who received gastrointestinal nutrition therapy.

RESULTS: Blood oxygen free radical level was significantly higher in parenteral nutrition group and regular treatment group compared with enteral nutrition group ($p< 0.05$). C-reactive protein and IL-6 contents were significantly reduced in parenteral nutrition group and regular treatment group compared to those in enteral nutrition group ($p< 0.05$). The expression of Bim at both mRNA and protein levels was declined in elderly patients with uremia after enteral nutrition combined with dialysis therapy to the normal level. The level of Bim was positively correlated with the severity of elderly uremia.

CONCLUSIONS: Bim is positively correlated with the severity of elderly uremia, which can be set as a potential specific biomarker, along with reactive oxygen radicals, CRP, IL-6, for the prognosis of elderly uremia.

Key Words:
Elderly uremia, Dialysis, Bim, Diagnosis.

Introduction

Elderly uremia signifies the end stage of various chronic kidney diseases (CKD). As one of the difficult diseases in the urinary system, it is featured with high incidence and mortality[1]. The theraphy of uremia includes medical treatment, organ transplantation, and dialysis[2,3]. The above-mentioned therapies have played an important key role, but still present several shortcomings and deficiencies[4,5]. For instance, traditional medical treatment works slowly; the donors for elderly uremia are scarce, which is incapable to satisfy the actual demand[6]. Therefore, dialysis represents one of the commonly used methods for the treatment of uremia. However, blood dialysis is limited by malnutrition, immune suppression, and toxic oxygen ions, which seriously affects the quality of life and interferes prognosis of elderly patients with uremia[7,8].

In recent years, enteral nutrition is often used in uremia patients receiving dialysis and exhibits significant curative effect[9]. Enteral nutrition mainly exerts function by providing the necessary nutrients through the intestinal tract. More importantly, enteral nutrition diminishes the side effects caused by dialysis, and it thus reduces the complications and mortality[10]. The early diagnostic molecular biomarkers of elderly uremia mainly include renal function index, intestinal function index, and inflammatory factors, etc. However, all of these molecular biomarkers exhibit unsatisfactory specificity and low detection accuracy[11,12]. Thus, ideal molecular biomarkers are urgently needed for elderly patients with uremia

Corresponding Author: Yihua Bai, MD; e-mail: kuesuqgsssowig@sina.com

received gastrointestinal nutrition combined with dialysis in clinical practice[13]. Bim is a BH3-only subfamily protein belonging to the Bcl-2 family. Bim serves as the key factor to induce apoptosis, such as in neurons and keratinocytes[8,10]. Bim induces cell apoptosis caused by oxidative stress, while cell apoptosis is inhibited in Bim knockout cells[12,14,15]. Mitochondrial protein Bim contributes to regulating inflammation, restraining oxidative stress, and maintaining the balance of the mitochondrial membrane potential and energy production[14]. The role of Bim in elderly uremia remains to be further discussed, for there is no clear evidence on the relationship between Bim and elderly uremia who received gastrointestinal nutrition with dialysis[15]. In this study, we detected Bim expression in elderly patients with uremia and analyzed the correlation between Bim level and the prognosis of elderly uremia.

Patients and Methods

Experimental Cases

According to the inclusion and exclusion criteria[16,17], a total of 312 elderly patients with uremia received gastrointestinal nutrition combined with dialysis therapy between Feb 2014 and Feb 2016 were enrolled in the Second Affiliated Hospital of Kunming Medical University, including 138 cases of elderly patients with uremia received gastrointestinal nutrition combined with dialysis therapy and 174 cases of elderly patients with uremia received severe gastrointestinal nutrition combined with dialysis therapy. Another 138 cases were selected as control. Blood sample before and after gastrointestinal nutrition therapy combined with dialysis was collected. This study was approved by the Ethics Committee of the Second Affiliated Hospital of Kunming Medical University. All patients enrolled in this study signed the informed consents after a detailed explanation.

Elderly patients with uremia in our hospital were selected and divided into parenteral nutrition group, enteral nutrition group, and regular treatment group. The regular treatment group received dialysis treatment at 2-4 times per week. The parenteral nutrition group received parenteral nutrition therapy except for dialysis. The enteral nutrition group received enteral nutrition therapy except for dialysis. The mean age was 54.4 ± 17.5 (15-83) years old in 312 cases of elderly uremia, while it was 59.2 ± 18.3 (20-84) years old in the control. No statistical difference was observed between two groups.

Blood Sample Collection

The blood was extracted and centrifuged. The precipitated blood cells were resuspended in 3.8% sodium citrate solution for the following experiments.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted using TRIzol[18] (Invitrogen, Carlsbad, CA, USA) and used for RT-PCR (TaKaRa, Otsu, Shiga, Japan). The primer sequences were as follows: Bim, forward, 5'-GATAGCCTCTCTTACTACC-3'; reverse, 5'-ACTGAGGGGCTCTGGTCTGCG-3'. Actin, forward, 5'-AAGAGGCTCACCCTGTCG-3'; reverse, 5'-TCAACGTTGTTGTCCATGGACG3'.

Agarose Gel Detection

Agarose gel electrophoresis was applied to detect the product of RT-PCR according to the manual[19]. E-gel imager system was used to analyze the gel (Bio-rad, Hercules, CA, USA).

Flow Cytometry

Reactive oxygen species-specific dye DCF (Beyotime, Beijing, China) was used to test reactive oxygen species level in the blood[20]. The blood suspension was treated by DCF at 0.001 mM for 30 min. The exciting light wavelength and absorption light wavelength were 488 nm and 625 nm, respectively.

Western Blot

Proteins were resolved on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Western blot was performed starting with 60 V electrophoresis for 30 min, followed by 120 V electrophoresis for 120 min. After electrophoresis, proteins were transferred to polyvinylidene difluoride (PVDF) membrane under 300 mA for 30 min. The membrane was then blocked with 5% defatted milk powder for 60 min at room temperature. Mouse anti-human CRP, IL-6, Bim, and β-actin antibody (all diluted at 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) were added for 4°C room temperature incubation overnight. The membrane was then washed with phosphate buffered-solution and Tween 20 (PBST) for 30 min, followed with incubation with secondary antibody for 60 min (1:5000, Santa Cruz Biotechnology, Santa Cruz, CA, USA). After washed three times with PBS-T, chemiluminescence detection reagent was used to develop and fix. Gel image system was used to analyze the band density (Bio-Rad, Hercules, CA, USA)[21].

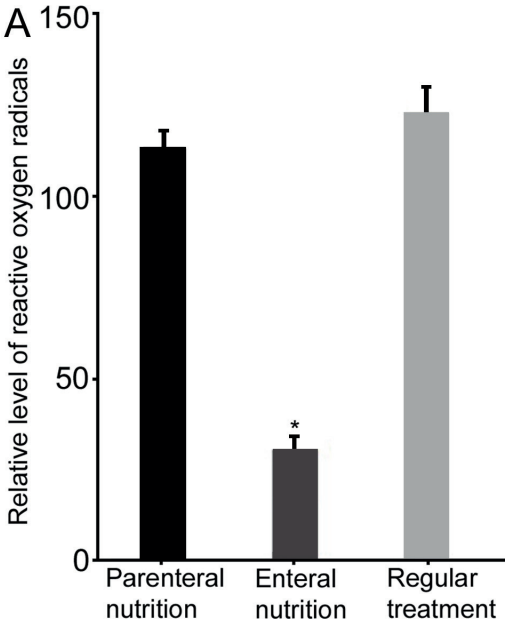

Figure 1. Reactive oxygen radical detection in blood from elderly uremia. *$p< 0.05$, compared with regulator treatment group.

Statistical Analysis

All the data analysis was performed on SPSS 22.0 software (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA)[22]. The data was analyzed by normal distribution and presented as mean ± standard deviation. The *t*-test was used for the intergroup comparison. Continuous data from multiple groups were analyzed by using one-way ANOVA, with the Tukey's post-hoc test. $p<0.05$ was considered as statistical significant.

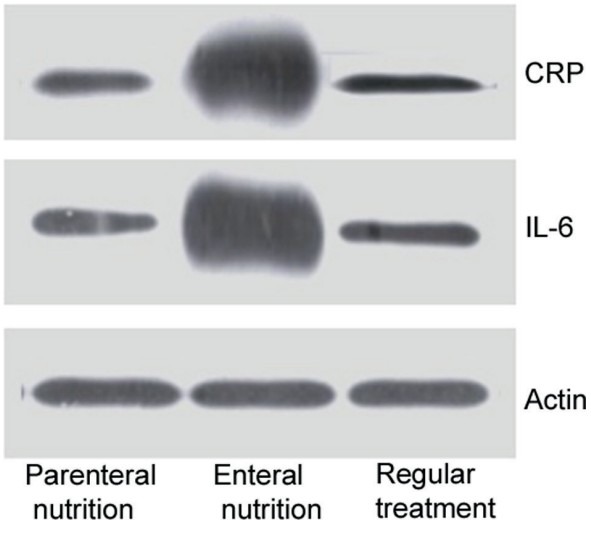

Figure 2. C-reactive protein and IL-6 detection in blood from elderly uremia by Western blotting

Results

Reactive Oxygen Radicals Detection

As shown in Figure 1, flow cytometry detection indicated that blood oxygen free radical level was significantly higher in parenteral nutrition group and regular treatment group compared with that in enteral nutrition group ($p< 0.05$). No statistical difference of blood oxygen free radical level was observed between parenteral nutrition group and control group.

Immune Function Index Detection

Western blot data revealed that C-reactive protein and IL-6 contents were significantly lower in parenteral nutrition group and regular treatment group than that in enteral nutrition group (Figure 2).

Bim Protein Detection

Also, our result demonstrated that Bim protein level was markedly declined in elderly patients with uremia after enteral nutrition combined with dialysis therapy, compared to the normal level. However, no evident difference was found between parenteral nutrition group and regular treatment group (Figure 3).

Bim mRNA Expression Detection

Similarly, RT-PCR result indicated that Bim mRNA level was also significantly reduced in patients from enteral nutrition group ($p< 0.05$).

Correlation Analysis of Bim and Elderly Patients With Uremia Received Gastrointestinal Nutrition Combined With Dialysis

As shown in Figure 5, Bim was positively correlated with the severity of elderly uremia. Enteral nutrition can improve the prognosis by reducing oxygen free radical, improving immune state, and downregulating Bim level.

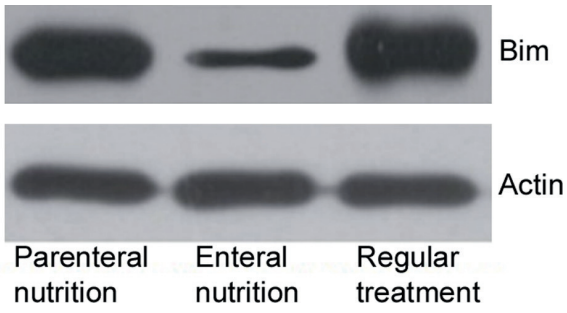

Figure 3. Bim protein detection in blood from elderly uremia by Western blotting.

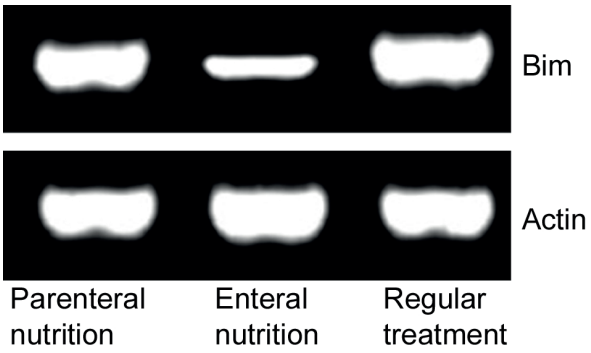

Figure 4. Bim mRNA detection in blood from elderly uremia.

Discussion

Effective diagnosis and treatment of gastrointestinal nutrition combined with dialysis in elderly uremia are extremely important[23,24]. However, the efficacy of molecular biomarkers for the clinical application of gastrointestinal nutrition combined with dialysis in elderly uremia remains unsatifactory[25]. Elderly uremia treated by gastrointestinal nutrition combined with dialysis forms a chronic inflammatory process induced by infection. CRP and IL-6 cause immunologic derangement and become one of the major reasons for the complications of elderly uremia received gastrointestinal nutrition combined with dialysis[26,27]. At present, the molecular markers for diagnosis of complications in elderly uremia are still in the process of basic research.

Bim upregulation can significantly decrease the mitochondrial membrane potential[28]. Under physiological and pathological conditions, Bim directly or indirectly modulates mitochondrial membrane potential, and also significantly increases the mitochondrial calcium overload, elevates the level of active oxygen free radicals, and promotes cell apoptosis. Of note, our study showed that the expression of Bim at both mRNA and protein levels was declined in elderly patients with uremia after enteral nutrition combined with dialysis therapy to the normal level. Also, enteral nutrition apparently decreased blood oxygen free radical level, but markedly elevated the expressions of CRP and IL-6, indicating Bim, as well as oxygen free radical level, CRP and IL-6 were affected by enteral nutrition treatment in elderly patients with uremia after enteral nutrition combined with dialysis therapy. Moreover, Bim was positively correlated with the severity of elderly uremia, suggesting that Bim can serve as the indicator for the prognosis[26-28]. However, the role of Bim in the diagnosis of elderly uremia needs further evaluation within a large amount of samples in practice. Uremia combined malnutrition may exhibit immunodeficiency and dialysis-related chronic complications, seriously influences the quality of life and prognosis of elderly patients with uremia. Notably, our result showed critical role of enteral nutrition in the improvement of elderly uremia, which was in agreement with previous finding of its contribution in maintenance and support of intestinal mucosal barrier, promoting intestinal peristalsis, improving tissue perfusion and nutritional intake, and significantly reducing infectious complications and the fatality rate[29], although the practical efficacy as well as the clinical application requires in-depth investigation.

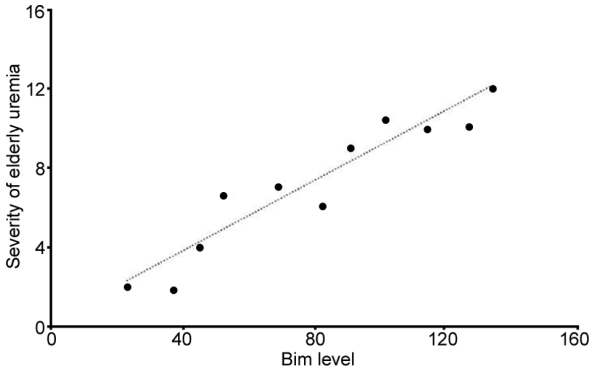

Figure 5. Correlation analysis of Bim and elderly patients with uremia received gastrointestinal nutrition combined with dialysis.

Conclusions

We demostrated that serum Bim was positively correlated with the severity of elderly uremia, which can be set as a potential specific biomarker, along with reactive oxygen radicals, CRP, IL-6, for the prognosis of elderly uremia.

Acknowledgments

This work was supported by Project of Science and Technology Department of Yunnan Province (2015FB054).

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Crespo-Salgado J, Vehaskari VM, Stewart T, Ferris M, Zhang Q, Wang G, Blanchard EE, Taylor CM, Kallash M, Greenbaum LA, Aviles DH. Intestinal microbiota in pediatric patients with end stage renal disease: a Midwest Pediatric Nephrology Consortium study. Microbiome 2016; 4: 50.
2) Cetin N, Sav NM, Karabel D, Yildirim A, Yildiz B. Serum albumin and von Willebrand factor: possible markers for early detection of vascular damage in children undergoing peritoneal dialysis. Clin Invest Med 2016; 39: E111-119.
3) Zhang W, Liu J, Sun R, Zhao L, Du J, Ruan C, Dai K. Calpain activator dibucaine induces platelet apoptosis. Int J Mol Sci 2011; 12: 2125-2137.
4) Schonermarck U, Dengler C, Gmeinwieser A, Praun S, Schelling G, Fischereder M, Boulesteix AL, Dolch ME. Exhaled breath volatile organic and inorganic compound composition in end-stage renal disease. Clin Nephrol 2016; 86: 132-140.
5) Djordjevic S, Kitic D, Kostic M, Apostolovic B, Brankovic S, Ciric IM, Velickovic-Radovanovic R. The joint influence of protein supplements, soft drinks and extreme physical activity on the development of acute renal failure and hypokalaemia. West Indian Med J 2017; 66: 376-379.
6) Bucar Pajek M, Leskosek B, Vivoda T, Svilan K, Cuk I, Pajek J. Integrative examination of motor abilities in dialysis patients and selection of tests for a standardized physical function assessment. Ther Apher Dial 2016; 20: 286-294.
7) Zhang W, Zhao L, Liu J, Du J, Wang Z, Ruan C, Dai K. Cisplatin induces platelet apoptosis through the ERK signaling pathway. Thromb Res 2012; 130: 81-91.
8) Basu B, Mahapatra TK, Roy B, Schaefer F. Efficacy and outcomes of continuous peritoneal dialysis versus daily intermittent hemodialysis in pediatric acute kidney injury. Pediatr Nephrol 2016; 31: 1681-1689.
9) Desai N, Rahman M. Nephrology update: end-stage renal disease and renal replacement therapy. FP Essent 2016; 444: 23-29.
10) Perna AF, Di Nunzio A, Amoresano A, Pane F, Fontanarosa C, Pucci P, Vigorito C, Cirillo G, Zacchia M, Trepiccione F, Ingrosso D. Divergent behavior of hydrogen sulfide pools and of the sulfur metabolite lanthionine, a novel uremic toxin, in dialysis patients. Biochimie 2016; 126: 97-107.
11) Gandhi K, Prasad D, Malhotra V, Agrawal D, Beniwal P, Mathur M. Periodic peritoneal dialysis in end stage renal disease: is it still relevant? A single center study from India. Ann Med Health Sci Res 2015; 5: 379-384.
12) Aatif T, El Farouki MR, Benyahia M. Posterior reversible encephalopathy syndrome in a hypertensive patient with renal failure. Saudi J Kidney Dis Transpl 2016; 27: 411-414.
13) Gonzalez E, Diez JJ, Bajo MA, del Peso G, Grande C, Rodriguez O, Diaz-Almiron M, Iglesias P, Selgas R. Fibroblast Growth Factor 21 (FGF-21) in peritoneal dialysis patients: natural history and metabolic implications. PLoS One 2016; 11: e0151698.
14) Wu HY, Peng YS, Chen HY, Tsai WC, Yang JY, Hsu SP, Pai MF, Lu HM, Chiang JF, Ko MJ, Wen SY, Chiu HC. A comparison of uremic pruritus in patients receiving peritoneal dialysis and hemodialysis. Medicine (Baltimore) 2016; 95: e2935.
15) Dias DB, Banin V, Mendes ML, Barretti P, Ponce D. Peritoneal dialysis can be an option for unplanned chronic dialysis: initial results from a developing country. Int Urol Nephrol 2016; 48: 901-906.
16) Kovesdy CP, Kalantar-Zadeh K. Back to the future: restricted protein intake for conservative management of CKD, triple goals of renoprotection, uremia mitigation, and nutritional health. Int Urol Nephrol 2016; 48: 725-729.
17) Akbari R, Najafi I, Maleki S, Alizadeh-Navaei R. Serum mannose-binding lectin in patients on peritoneal dialysis compared with healthy individuals. Iran J Kidney Dis 2016; 10: 26-29.
18) Sarmento-Dias M, Santos-Araujo C, Poinhos R, Oliveira B, Silva IS, Silva LS, Sousa MJ, Correia F, Pestana M. Fibroblast growth factor 23 is associated with left ventricular hypertrophy, not with uremic vasculopathy in peritoneal dialysis patients. Clin Nephrol 2016; 85: 135-141.
19) Wong SP, Vig EK, Taylor JS, Burrows NR, Liu CF, Williams DE, Hebert PL, O'Hare AM. Timing of initiation of maintenance dialysis: a qualitative analysis of the electronic medical records of a national cohort of patients from the Department of Veterans Affairs. JAMA Intern Med 2016; 176: 228-235.
20) Chang CT, Wang GJ, Kuo CC, Hsieh JY, Lee AS, Chang CM, Wang CC, Shen MY, Huang CC, Sawamura T, Yang CY, Stancel N, Chen CH. Electronegative low-density lipoprotein increases coronary artery disease risk in uremia patients on maintenance hemodialysis. Medicine (Baltimore) 2016; 95: e2265.
21) Nacak H, Bolignano D, Van Diepen M, Dekker F, Van Biesen W. Timing of start of dialysis in diabetes mellitus patients: a systematic literature review. Nephrol Dial Transplant 2016; 31: 306-316.
22) Iwafuchi Y, Okamoto K, Oyama Y, Narita I. Posterior reversible encephalopathy syndrome in a patient with severe uremia without hypertension. Intern Med 2016; 55: 63-68.
23) Ferrantelli E, Liappas G, Keuning ED, Vila Cuenca M, Gonzalez-Mateo G, Verkaik M, Lopez-Cabrera M, Beelen RH. A novel mouse model of peritoneal dialysis: combination of uraemia and long-term exposure to PD fluid. Biomed Res Int 2015; 2015: 106902.
24) Gonzalez E, Bajo MA, Carrero JJ, Lindholm B, Grande C, Sanchez-Villanueva R, Del Peso G, Diaz-Almiron M, Iglesias P, Diez JJ, Selgas R. An increase of plasma advanced oxidation protein products levels is associated with cardiovascular risk in incident peritoneal dialysis patients: a pilot study. Oxid Med Cell Longev 2015; 2015: 219569.
25) Li J, Guo Q, Lin J, Yi C, Yang X, Yu X. Prevalence and associated factors of uraemic pruritus in con-

tinuous ambulatory peritoneal dialysis patients. Intern Med 2015; 54: 2827-2833.

26) Wang SJ, Qu ZS, Zhang QD, Li L, Wang F, Zhang B, Wu BL, Zhao YW. The serum levels of MMP-9, MMP-2 and vWF in patients with low doses of urokinase peritoneal dialysis decreased uremia complicated with cerebral infarction. Int J Clin Exp Med 2015; 8: 13017-13027.

27) Xiang FF, Zhu JM, Cao XS, Shen B, Zou JZ, Liu ZH, Zhang H, Teng J, Liu H, Ding XQ. Lymphocyte depletion and subset alteration correlate to renal function in chronic kidney disease patients. Ren Fail 2016; 38: 7-14.

28) Sun L, Liu H, Jiang H, Wei M, Liang S, Wang M, Shi K, He Q. Macrophages are involved in gut bacterial translocation and reversed by lactobacillus in experimental uremia. Dig Dis Sci 2016; 61: 1534-1544.

29) Shen QX, Xu GX, Shen MH. Effect of early enteral nutrition (EN) on endotoxin in serum and intestinal permeability in patients with severe acute pancreatitis. Eur Rev Med Pharmacol Sci 2017; 21: 2764-2768.