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Original Article

Influence of colonic dialysis using Chinese medicine on creatinine decomposition by intestinal bacteria in uremia rats

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Abstract: Objectives: This study aims to investigate influence of colonic dialysis using Chinese medicine on creatinine decomposition by intestinal bacteria in uremia rats. Methods: Healthy male Sprague Dawley (SD) rats were randomly divided into three groups, including uremic group, sham group and Chinese medicinal group. Uremic model was established in uremic group and Chinese medicinal groupby 5/6 nephrectomy, while sham group did not undergo nephrectomy. All rats were sacrificed at the end of ten weeks. Serum creatinine was examined. Histopathological changes of rat kidney were observed by hematoxylin-eosin staining. The numbers of Bifidobacterium, Lactobacillus and E.co*li* in the intestinal tract were quantitatively determined by real-time fluorescent quantitative PCR with 16S RNA. Results: Compared with sham group, the number of *E.coli* in the jejunum increased significantly, while that of Bifidobacterium and Lactobaeillus in the ileum decreased. The number of Bifidobacterium and Lactobaeillus was decreased in the colon whereas that of *E.coli* increased. Conclusion: Our findings revealed that Influence of colonic dialysis using Chinese medicine on creatinine decomposition by intestinal bacteria in uremia rats.

Keywords: Uremia, Chinese medicine, creatinine decomposition, intestinal bacteria

Introduction

Uremia is a life-threatening disease characterized by renal failure, which means the kidney loses the function of detoxification and cannot produce urine. Numerous recent studied indicate that the microbiome dysbiosis of intestinal flora in uremic rats is closely related with microinflammation. With maturity and development of Chinese medicine technologies, more and more patients with uremia select hemodialysis with Chinese medicine. With the improvement of living standards, especially since the end of the 20st century, uremia has become one of the global health problems. Uremia is the end-stage change of chronic kidney disease, and the chronic kidney disease caused by diabetic nephropathy, hypertensive nephropathy and glomerular disease will eventually progress to end-stage renal failure and requires hemodialysis therapy. Uremia is one of the most common malignant digestive urinary system diseases, which means the kidney loses the function of detoxification and cannot produce urine [1, 2]. According to the International Society of Nephrology (ISN) statistics, in 2011, the Chinese patients with uremia has reached 200 million, and, by 2030, this figure is estimated to be 400 million [3].

Because of decline in the ability for scavenging, the blood concentration of metabolites in patients with uremia is increased. The increased toxins (such as urea, creatinine and uric acid) products provide good development situation for microorganism from gastrointestinal mucous membrane to the inner lumen [4]. Several previous studies has been found that blood from uremia patients and rats can be detected some bacteria that is highly homologous to gut bacteria [5, 6]. The gut bacteria in uremia are involved in the occurrence and development of microinflammation. The urinary tract infection rate of uremic patients was high and the drug resistance rates of pathogens were serious.

With maturity and development of hemodialysis technologies, more and more patients with ure-

mia select hemodialysis [7]. Low-flux hemodialysis is the most commonly used method for clinical hemodialysis at present, it removes the by-products of metabolism in the way of pure dispersion, its effect is ideal on removing the macromolecular toxins, but the effect is limited on removing the middle molecular and low molecular toxins, and it can't effectively correct the calcium-phosphorus metabolism disorders and microinflammatory state in the development and change of uremia [7]. High-flux hemodialysis is a new way of dialysis developed in recent years, which combines three removing methods: diffusion, convection and adsorption, and has strong removing effect on macromolecular and middle molecular toxins [8].

Chinese traditional medicine is floorboard of the traditional medicine, which is the important component of traditional Chinese medicine, has devoted to human being healthy [9, 10]. Traditional Chinese Medicine is key to the Chinese Pharmaceutical Industry, it shares about 40% of the pharmaceutical market. A large number of studies have suggested that the Chinese traditional medicine could improve level of cellular immunity, enhance non-specific immunological function and promote growth of the immune organs. With maturity and development of Chinese medicine technologies, more and more patients with uremia select hemodialysis with Chinese medicine. This study aims to investigate influence of colonic dialysis using Chinese medicine on creatinine decomposition by intestinal bacteria in uremia rats.

Materials and methods

Animals

90 male Sprague-Dawley (SD) rats (Experimental Animal Center of Minhang District Central Hospital) weighing 100-175 g were included in the study. All animals were kept at a constant room temperature of 18-22°C with a humidity of 70% and exposed to a 12/12 h light/dark cycle. All animals had access to water and standard rodent chow. The study was carried out, in agreement with the guidelines for animal research, according to the Guide for the Care and Use of Laboratory Animals published by the ethics and research committee of our hospital. The study protocol was reviewed and approved by the institutional animal care committee according to the Animal Protection Act of Minhang District Central Hospital.

The establishment of uremia model

According with the method published in 1986, the uremia model was established with feeding 0.75% adenine. Renal insufficiency was induced by 5/6 nephrectomy in a group of male SD rats weighting 250~300 g [11]. The 5/6 nephrectomy involves the ligation of several branches of the left renal artery and excision of the right kidney. After 7 weeks, the uremic rats were treated by using homemade drain tube line tube insertion, 25 ml/times, 4 times one day. The uremic rats were randomly divided into three groups (n = 30): uremic group, sham group and Chinese medicinal group. The ingredients of Chinese medicine: Rhubarb 30 g, practice of lateral 10 g, *Radix Ginseng Rubra* 10 g, *Holothuroidea* 50 g, *Carthamus tinctorius L.* 10 g, and *Taraxacum mongolicum Hand.-Mazz.* 30 g.

Histologic examination

Kidney tissue was embedded in paraffin, sectioned, and stained with HE-staining. Glomeruli exhibited adhesion of the capillary and tufted to the Bowman's capsule, capillary obliteration, mesangial expansion. The extent of glomerular damage was expressed as the percentage of glomeruli. For the extent of renal interstitial expansion, the fraction of renal cortex was occupied by interstitium. For extracellular matrix components by HE-staining was quantitatively evaluated by a point-counting technique in 10 randomly selected microscopic fields, at a final magnification of ×200 under a 100-point grid.

Biochemical identification for intestinal flora

0.5 g feces of uremia rat among each group were collected and weighed on electronic balance from the initial drug intervention, intervention in 4 weeks, 8 weeks. These feces were diluted with 4.5 ml saline with shaking and were moved into displacement, blood AGAR, macconkey AGAR plate culture medium and SS AGAR medium. According to a district scribe inoculated, we used sterile needle to vaccinate at 37°C incubator and checked the result after 24 h. The biochemical identification for intestinal flora was analyzed by Potassium hydroxide wire drawing test and gram stain.

RNA extraction and real-time quantitative polymerase chain reaction

Total RNA from tissues was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA), as per the manufacturer's instructions. 200 ng of RNA was reverse-transcribed to cDNA using RT2 First Strand Kit (Qiagen, 330401). Real-time PCR was performed on ABI Q6 detection system (Applied Biosystems Inc., USA) using the Real Time SYBR master mix kit (Qiagen, 337230).

The qualitative research for creatinine

The normal intestinal bacteria of each uremic rat from three experiment groups were equipped with 1 mL creatinine standby liquid aseptic in 1.5 mL Eppendor tube. About 10^8 colony-forming unit (cfu)/mL bacteria content was removed to 96-well cell culture plate with 180 μL micro pipette, and then added 20 μL 0.01% Tetrazolium in each well. Blank with physiological saline was as a negative control. The cell culture was plated in 37°C incubator, and was observed after 24 h. According to the above method, 1.5 mL Ep tube with no protein medium containing creatinine 900 μl bacteria suspension was added 100 μL 0.01% Tetrazolium violet solution in 37°C incubator for 24 h. Supernatant were measured on ultraviolet spectrophotometer in 280-310 nm absorbance measurement values.

The quantitative research for creatinine

After shaking with sterile syringe, sterile configuration with 0.14 mg/mL creatinine solution and 3 mL nutrient broth medium in each tube were packed in 5 mL centrifuge tube. Determination of creatinine levels for various culture was contrasted the value of the former. The intestinal bacteria culture identification of uremia rats with normal one annulus were picked respectively, added into the packaging 5 mL centrifuge tube, blended in the vortex oscillators in 37°C incubator for 24 h. Automatic biochemical detector blind after training to measure creatinine concentration in culture medium.

The separation and purification of creatinine enzyme

Bacteria in 9 cm agarose tablet was picked in a single colony respectively in protein free medium cultivation of 16-17 h, which contained 1200 μmol/L creatinine with no protein medium in 37°C oscillator 250 r/min for continuously culturing 48 h. The containing was 2400 μmol/L creatinine with no protein medium under the condition of same culture 48 h. We removed the liquid fermentation tank, centrifuged bacteria, joined the buffer, and centrifuged with whole automatic high-speed 7000 r/min for 10 min, All that a moderate amount of supernatant fluid, add with creatinine Tetrazolium violet solution (V: V/9:1), 37°C purple solution after about three hours. This was a qualitative test for creatinine enzyme decomposition, which proved the supernatant fluid creatinine enzyme exists. After heat preservation at 60°C for 30 min, solution was added with Tetrazolium violet. The thermal stability of the enzyme was good. The thick enzyme fluid heat preservation was processed in 55°C water bath for 30 min, the centrifugal removing protein thermal instability. Description creatinine enzyme in ammonium sulfate saturation for most settle was between 40-50%. 10% polyacrylamide gel electrophoresis of proteins was selected according to the molecular weight of 28.4 KD creatinine hydrolase.

The gene identification and presumption of amino acids about creatinine enzyme

According to the height gene homology of creatinine enzyme with DNA sequence of pseudomonas creatinine enzyme, design a pair of primers: F 5'-ATGAACGATAGCGTTGTAAT-3', R 5'-CTAACTGAATGCCTCGCGGA-3'. Amplification process: 94°C modified 5 min, take the PCR reaction conditions, a total of 38 cycles. According to the results of sequencing, the presumption of creatinine possible amino acid sequence of enzyme was analyzed.

Statistical analysis

The statistical analyses were performed using SPSS (Version 19.0; IBM, Armonk, NY, USA). Data was presented as mean ± SD (standard deviation) from three independent experiments with each measured in triplicate. A value of $P < 0.05$ was considered to be a statistically significant difference.

Discussion

Based on the changes in serum urea nitrogen, creatinine, residual renal pathological changes,

the uremia standard model was successfully built by this experiment 5/6 nephrectomy modeling method. On the basis of the observed in HE-staining, the Chinese medicine had some therapeutic effect on kidney damage in uremia rats.

From the stomach to the colon, the intestinal bacteria increased gradually. The stomach had only a small amount of acid bacteria and aerobic bacteria, and acid bacteria and aerobic bacteria increased obviously in intestine and colon. High bacteria and anaerobic bacteria accounted for more than 98% in colon [12, 13]. In normal condition, intestinal normal flora between intestine and colon maintained at a fairly stable proportion, which might constitute the intestinal microorganisms. However, in certain disease states (such as severe liver diseases, burns, stress), the normal flora in the intestines changed significantly and the number and density decreased obviously [14, 15]. In this present study, the number of advantage intestinal bacteria strains in uremic rats changed obviously, which indicated the existence of intestinal flora imbalance in uremia rat.

This study found that compared with sham group and Chinese medicinal group, the number of bifidobacterium and lactobacillus was decreased, the number of *E.coli* was increased in uremia group. The change of the three bacteria in the ileum: compared with sham group and Chinese medicinal group, the number of bifidobacterium and lactobacillus was decreased, the number of *E.coli* was increased in uremia group. Three kinds of bacteria in the jejunum changes: compared with sham group and Chinese medicinal group, the number of *E.coli* was increased in uremia group, the number of bifidobacteria and lactobacilli decreased in uremia group. Analysis for the following reasons: probiotics and the intestinal adhesion [16]. Under normal circumstances, probiotics and the intestinal epithelial membrane cells were together to form membrane. *E.coli* were adhered to the intestine surface. Since the intestinal villus shortened and cellular structure damaged, the adhesion ability of probiotic bacteria and intestinal epithelial cells was damaged [17].

The intestinal tract normal flora planting, breeding and exclusive foreign bacteria cannot colonize the gut, advantage and breeding to shift, this is called “Engraftment resistance” [18, 19]. The advantages of the lactobacillus bacteria in the human gut engraftment, its acidic metabolites such as acetic acid, lactic acid, short chain fatty acid can reduce intestinal local pH within the spectrum antibacterial effect. These substances within the gut on *E.coli*, salmonella, bacteria etc can also reduce the corruption and the production of harmful substances and endotoxin. In addition, lactobacillus and *E.coli* might inhibit adhesion of intestinal epithelial cells [20].

Within the lumen urea nitrogen, muscle when uremia anhydride concentration significantly increased, and many have different degrees of digestion and absorption function. Within the lumen is reduced, resulting in protein and amino acid retention, bacterial enzymes promoted role with plenty of substrate, stimulated was given priority to with *E.coli*. This study showed that uremia group and control group in the jejunum anaerobic bacteria number was no significant difference, only showed the E.coli increased ($P < 0.05$), which due to chronic renal failure, upper gastrointestinal pH value. Environmental change was more suitable for the survival of *E.coli*.

In this present study, we aimed to investigate influence of colonic dialysis using Chinese medicine on creatinine decomposition by intestinal bacteria in uremia rats. We divided healthy male SD rats into three groups, including uremic group, sham group and Chinese medicinal group. Uremic model was established in uremic group and Chinese medicinal groupby 5/6 nephrectomy, while sham group did not undergo nephrectomy. Histopathological changes of rat kidney were observed by HE and Masson staining. The numbers of Bifidobacterium, Lactobacillus and *E.coli* in the intestinal tract were quantitatively determined by real-time fluorescent quantitative PCR with 16S RNA. Compared with sham group, the number of E.coli in the jejunum increased significantly ($P < 0.05$), while that of Bifidobacterium and Lactobaeillus in the ileum decreased ($P < 0.05$). The number of Bifidobacterium and Lactobaeillus was decreased in the colon whereas that of E.coli increased ($P < 0.05$). Our findings revealed that Influence of colonic dialysis using Chinese medicine on creatinine decomposition by intestinal bacteria in uremia rats.

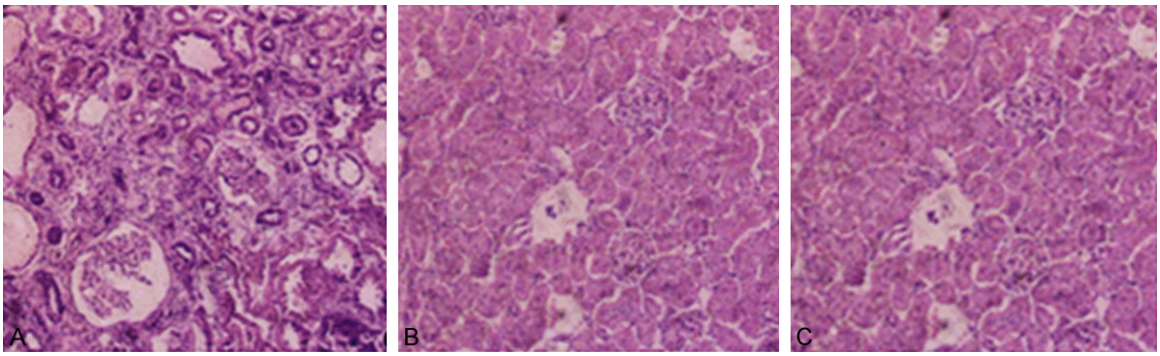

Figure 1. Renal histopathological changed in uremic group (A), sham group (B) and Chinese medicinal group (C) (×200) by HE-staining.

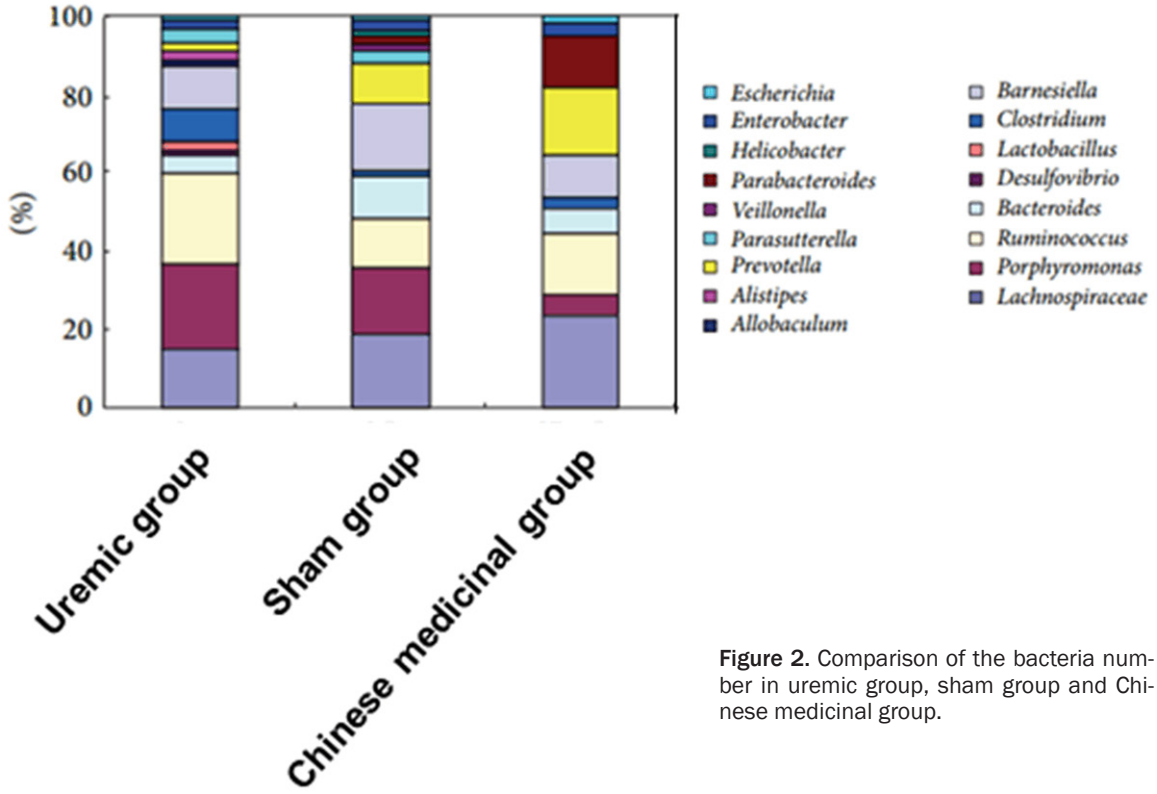

Figure 2. Comparison of the bacteria number in uremic group, sham group and Chinese medicinal group.

Conclusion

The Chinese medicine had some therapeutic effect on kidney damage in uremia rats

In uremia group, the glomerular structure of rat was disordered, the glomerular had focal or diffuse sclerosis, partly compensatory glomerular and the protein tube type was existed in renal small tube cavity. There were renal interstitial infiltration of inflammatory cells and fibrous tissue hyperplasia in uremia group (**Figure 1A**). In sham group and Chinese medicinal group, the rat glomerular structure was clear, open capillary loops, no obvious mesangial proliferation. Renal interstitial inflammatory cell infiltration and fibrous tissue hyperplasia were observed in sham group and Chinese medicinal group (**Figure 1B** and **1C**).

The Chinese medicine had some effects on intestinal flora in uremia rats

The change of some bacteria (such as bifidobacterium, lactobacillus, *E.coli*) in the colon: compared with sham group and Chinese medic-

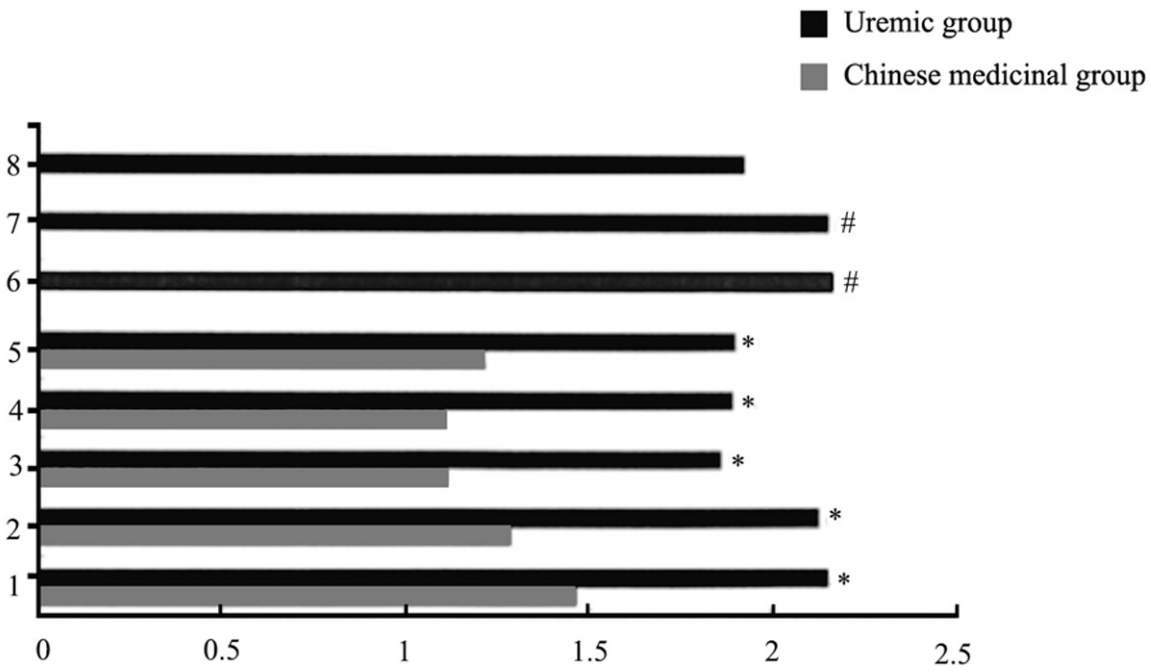

Figure 3. The absorbance in different inteseinal segment in uremic group and Chinese medicinal group, compared with the sham group.

inal group, the number of bifidobacterium and lactobacillus was decreased, the number of *E.coli* was increased in uremia group ($P < 0.05$). The change of the three bacteria in the ileum: compared with sham group and Chinese medicinal group, the number of bifidobacterium and lactobacillus was decreased ($P < 0.05$), the number of *E.coli* was increased in uremia group, but had no statistically significant difference ($P > 0.05$). Three kinds of bacteria in the jejunum changes: compared with sham group and Chinese medicinal group, the number of *E.coli* was increased in uremia group ($P < 0.05$), the number of bifidobacteria and lactobacilli was decreased in uremia group but the difference was not significant ($P > 0.05$, **Figure 2**).

The intestinal flora had some effects on creatinine decompose in uremia rats

The change of the three kinds of bacteria in the colon: compared with sham group and Chinese medicinal group, uremia group decreased in the number of bifidobacterium, lactobacillus, increased in the number of E.coli ($P < 0.05$).The change of the three kinds of bacteria in the ileum: compared with sham group and Chinese medicinal group, bifidobacterium and lactobacillus of uremia group were decreased ($P < 0.05$), the number of E.coli was increased, but had no statistically significant difference ($P > 0.05$). Three kinds of bacteria in the jejunum changes: compared with sham group and Chinese medicinal group, the number of *E.coli* was increased, the number of bifidobacteria and lactobacilli was decreased ($P > 0.05$), but the difference was not significant ($P > 0.05$, **Figure 3**).

The gene identification and presumption of amino acids about creatinine enzyme

These genes and Pseudomonas putida creatininase gene relatively, homology was above 98%; Amino acid sequence of pseudomonas putida creatininasc protein LI was more than 97% homology (**Figure 4**).

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Disclosure of conflict of interest

None.

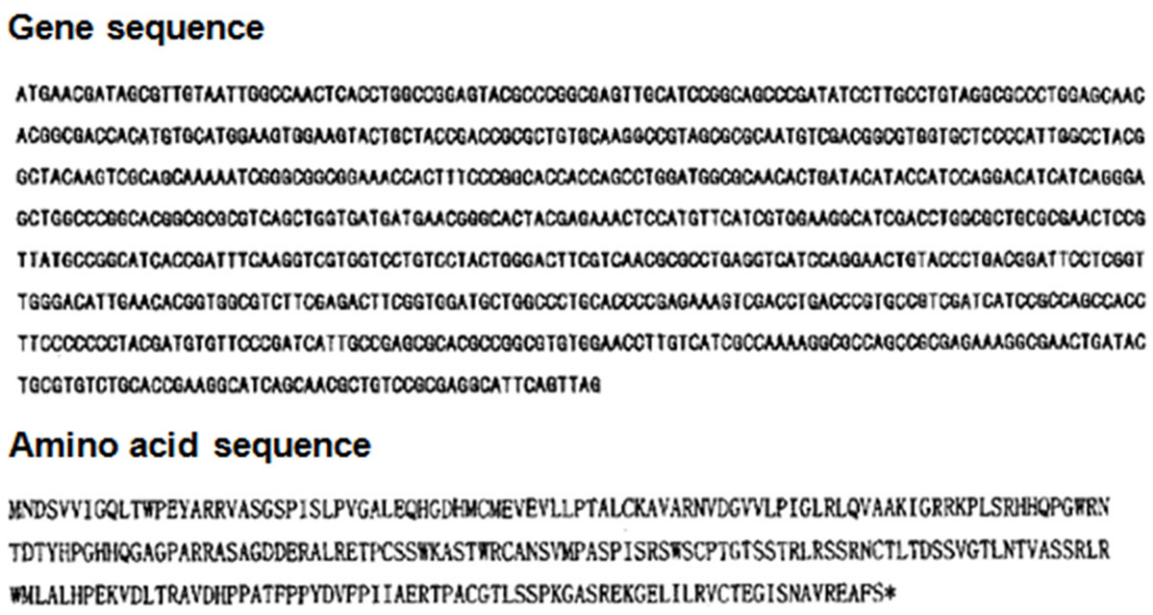

Figure 4. The gene identification and presumption of amino acids about Creatinine enzyme.

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