Cross-linked Polyelectrolyte and Its Function in Adsorption of Fluid and Excess Nitrogen Waste Products

An Experimental Study on Dialysate Effluent Fluid

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Introduction. One of the most important issues in patients with chronic kidney disease is fluid retention and volume overload accompanied by retention of nitrogenous waste products and some electrolytes. Bowel fluid contains high levels of urea, creatinine, uric acid, and electrolytes, which make it a potential candidate for intestinal excretion of nitrogen wastes and electrolytes. Cross-linked polyelectrolyte (CLP) is a polymer that, given orally, absorbs excess fluid, electrolyte, and nitrogenous waste products.

Materials and Methods. In an experimental study on 30 hemodialysis patients, the effect of CLP on adsorption of fluid, urea, creatinine, uric acid, sodium, and potassium were evaluated. For this purpose, 500 mL of effluent fluid of each patient were collected at the 1st hour of dialysis. The concentrations of the abovementioned products were measured by standard methods. Then the dialysate effluent samples were treated with 6 g of CLP and incubated for 4 hours at 37°C.

Results. Up to 80% of effluent fluid water was adsorbed by CLP. There were significant reductions in urea, creatinine, uric acid, and sodium levels in the remaining effluent fluid ($P < .001$). In contrast, the amount of potassium increased in the effluent fluid.

Conclusions. Using CLP in addition to functional medical super adsorbents can be a possible adequate substitute for conventional dialysis methods, especially hemodialysis.

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INTRODUCTION

Volume overload has been associated with increased prevalence of uncontrolled hypertension in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD) on long-term dialysis. Excessive interdialytic weight gain coupled with inability to achieve dry weight due to short dialysis times are some factors that potentially contribute to inadequate blood pressure (BP) control.[1,2] Fluid retention is a major clinical problem in patients with CKD at terminal stage and patients on dialysis.[3] Different dialysis modalities may have different effects on fluid volume control.[4] It has been shown that patients on long-term continues ambulatory peritoneal dialysis (CAPD) had more volume overload episodes than patients on short-term dialysis, which is explained by timely reduction of residual kidney function.[1]

On the other hand, fluid retention appears to be more important than hypertension and it has high predictive value of increased risk of adverse outcomes.[5] In addition, in a recent study, a high percentage of dialysis patients (39%) exhibited considerable fluid overload before hemodialysis.[6]

Under normal conditions, the kidney plays a major role in elimination of excess fluid, urea, and its metabolites. However, in patients with CKD, the intestine can substitute some function of the kidney in clearance of nitrogenous waste products and other toxins.[7] Gastrointestinal tract is the major pathway of entry of the fluid, electrolytes, and nitrogenous products into body. It has the potential of removing the fluid and waste products from the gut and substitution of the some excretory function of the kidney by the intestine in some patients with advance kidney disease and dialysis patients.[8] During the past decade, the use of enteral root including activated charcoal,[9] AST 120,[10] prebiotic, probiotics, [11] bentonite,[12] and induction of diarrhea have been introduced for intestinal elimination of nitrogenous waste products and reduction of the need for dialysis. Recent developments in polymer technology have generated materials with both uremia-specific solute and fluid adsorptive properties, which portend renewed opportunities for the clinical use of oral sorbents to ameliorate azotemia, fluid overload, and dialysis dependency of uremic animals.[13,14] These advances will provide medical approaches for renal replacement in patients with CKD and ESRD.[15]

Bowel fluid contains high levels of urea, creatinine, uric acid, and electrolytes, which makes it a potential candidate for intestinal excretion of nitrogen wastes and electrolytes. Intestinal fluids in a uremic patient daily contain 2.9 g of creatinine, 70 g of urea, and 2.5 g of uric acid.(22) A cross-link is a covalent bond or ionic bond that links one polymer chain to another. Polyelectrolytes are macromolecules that carrying either positively or negatively charged ionizable groups. Cross-linked polyelectrolyte (CLP) is a polymer that, given orally, absorbs excess fluid, electrolyte, and nitrogenous waste products in the gastrointestinal tract with eventual elimination in the faces.[15] The aim of the present study was to evaluate the safety and efficiency of CLP administration in adsorption and removal of excess fluid, electrolyte, and nitrogenous waste products in CKD patients. We predicted that this technique could be used as a potential adjuvant for conventional dialysis modalities including hemodialysis.

MATERIALS AND METHODS

Patients

Thirty patients (24 men and 6 women) with age range of 20 to 80 years participated in the present study. All of the patients had a history of CKD and were on dialysis at the hemodialysis center of a university hospital of Tabriz University of Medical Sciences, thrice weekly. Serum urea, creatinine, uric acid, sodium, and potassium concentrations of all patients were measured before dialysis (Table 1). All participants provided written informed consent prior to study participation.

Study Protocol

All patients were dialyzed using hemodialysis machines made by Fresenius Medical Care (Bad Homburg, Germany) with low-flux filter; F7 or F8 (Helal Iran Medical Devices Co; Soha, Karaj, Iran) or high-flux filters; F60 (Fresenius, Bad Homburg, Germany). During the first hour of hemodialysis, 500 mL of dialysate effluent fluid was collected from participants. Concentrations of urea, creatinine, uric acid, sodium, and potassium were measured in all samples before CLP treating (Table 1). In the next step, all of the dialysate effluent samples were treated with 6 g of CLP, and then incubated for 4 hours at 37°C. The swollen gel particles and the remaining fluid were separated using centrifugation. The concentrations of urea, creatinine, uric acid, sodium, and potassium were measured in remaining fluid (Table 1). The concentrations of nitrogen waste products and electrolytes were measured using a Hitachi 9.7 analyzer (Hitachi, Tokyo, Japan) and ion-selective electrode technique, respectively. The pH was measured using digital pH meter (BOECO pH meter PT-370, Germany). Finally, the amount of the abovementioned products, before and after CLP treating, were measured using the following equations[16]:

Amount in dialysate effluent before CLP = $V \times C1$

Amount in dialysate effluent after CLP = $V \times (1\text{-}A) \times C2$

Adsorbed amount = $(V \times C1) - [V \times (1 - A) \times C2]$

where V, A, C1, and C2 represent volume of dialysate effluent fluid, CLP adsorption percentage, and concentration of one especial product before and after CLP treating, respectively.

Hydrophilic Polymers

The CLP employed in the present study was Stockosorb 500 XL (Stockhausen, Krefeld, Germany), a cross-linked acrylamide/acrylic acid copolymer partially potassium neutralized. The granule

Table 1. Serum Urea, Creatinine, Uric Acid, Sodium, and Potassium Concentrations of All Patients

Patient	Age, y	Sex	Urea, mg/dL	Creatinine, mg/dL	Uric Acid, mg/dL	Sodium, mEq/L	Potassium, mEq/L
1	70	Female	83	3.26	9.2	140	4.0
2	36	Male	134	7.25	6.9	134	3.7
3	78	Male	132	7.09	7.3	136	4.6
4	62	Male	92	10.30	7.5	138	5.1
5	20	Female	59	4.26	6.5	137	6.4
6	57	Male	53	4.70	8.3	138	4.9
z	45	Male	65	6.52	8.2	135	4.8
8	36	Male	86	5.54	8.8	132	3.4
9	54	Male	84	7.25	7.1	141	4.5
10	55	Male	133	8.01	7.9	140	4.7
11	62	Male	84	5.68	6.6	142	4.9
12	60	Male	256	10.57	6.8	141	5.2
13	59	Male	49	4.35	8.3	136	4.7
14	47	Male	124	8.83	9.0	140	5.0
15	48	Male	78	5.25	8.5	130	5.2
16	80	Male	105	8.95	6.5	139	5.3
17	78	Male	140	9.78	5.9	132	5.0
18	60	Male	49	6.40	6.8	139	3.8
19	57	Male	61	5.02	8.1	137	4.6
20	60	Male	71	4.11	8.8	140	4.7
21	24	Female	82	6.83	7.4	134	4.4
22	63	Male	141	8.75	7.6	141	4.4
23	53	Male	65	5.75	5.6	129	5.8
24	58	Female	221	14.6	6.5	142	4.7
25	60	Female	140	7.13	7.7	142	4.8
26	26	Male	148	7.88	7.4	139	3.9
27	57	Male	227	11.55	8.2	138	4.7
28	43	Male	200	12.70	9.5	136	4.1
29	65	Female	144	4.35	8.7	133	4.4
30	67	Male	203	7.80	10.2	133	5.2

sizes ranged between 1.5 mm and 2.0 mm. When incubated at 37°C for 4 hours, the Stockosorb 500 XL can adsorb up to 400% of its weight in distilled water and up to 80% of its weight in dialysate effluent fluid. In a desired CLP product with ideal properties, the starting toxic monomers should be converted to nontoxic products via polymerization processes. According to the MTT test, Stockosorb 500 XL is a nontoxic polymer, which means that the conversion of monomer to polymer is nearly 100% in this polymer.

MTT Test

To explore the potential applications of hydrophilic polymer prior to clinical studies, the evaluation of its cellular compatibility and cytotoxicity were performed. The MTT assay was applied according to our previous studies.[17,18] Briefly, subcutaneous adipose tissue-derived mesenchymal stem cells were obtained and characterized using our established protocols.[19,20] Passage 3 of cells were used for experiments. Adipose tissue-derived mesenchymal stem cells were seeded in the 96-well plates at a density of 6000 cells per well. After 24 hours incubation at 37°C, 5% carbon dioxide, humid atmosphere, the cells were treated with different doses of polymer for 72 hours. Then, the medium containing polymers were removed and replaced with 2 mg/mL of MTT solution (Sigma), incubated for 4 hours in incubator. Next, the formazan crystals formed in the bottom of wells were dissolved in dimethyl sulfoxide (Sigma) and subjected to enzyme-linked immunosorbent assay reader for absorbance reading at 570 nm.

Statistical Analyses

All experiments were done in triplicate, and data were reported as mean ± standard deviation. The 1-way analysis of variance was used for comparing

the means of MTT assay, and the paired *t* test was used for analyzing the significance in dialysate effluent samples before and after CLP treating. The SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, IL, USA) was used for the analyses. *P* values less than .05 were considered significant.

RESULTS

The adsorbing percentages of effluent samples on CLP network are presented in Table 2. As it is clear, CLP can adsorb up to 0.78 times its weight in dialysate effluent fluid (81.00 ± 0.02%). The concentrations of creatinine, urea, uric acid, sodium, and potassium in the dialysate effluent samples, before and after CLP treating, are presented in Table 2.

By using these concentrations and the adsorption percentage of dialysate effluent fluid on CLP network, the amount of these products were computed in a given volume of effluent fluid. The results show a significant decrease in the amount of urea, creatinine, uric acid, and sodium after CLP treating ($P < .001$; Figures 1 to 4). In contrast, the amount of potassium increased after CLP treating (Figure 5).

The mean adsorption percentage of urea, creatinine, uric acid, and sodium were 50.00 ± 0.11, 80.00 ± 0.10, 76.00 ± 0.03, and 83.00 ± 0.02, respectively. Surprisingly, in most of the patients, potassium was not only adsorbed by CLP network but also efficiently transferred from CLP into the dialysate effluent fluid. The mean amount of potassium before and after CLP treating was 1.43 ± 0.44 mEq and 3.77 ± 1.53 mEq, respectively.

Table 2. Concentrations of Urea, Creatinine, Uric Acid, Sodium, and Potassium Before and After Treating Effluent Samples With Cross-linked Polyelectrolyte

Patient	Adsorbing*	Urea, mg/dL		Creatinine, mg/dL		Uric Acid, mg/dL		Sodium, mEq/L		Potassium, mEq/L	
		Before	After	Before	After	Before	After	Before	After	Before	After
1	0.81	22.0	60.0	0.81	0.82	1.0	1.4	128.8	107.9	3.84	27.48
2	0.79	24.0	59.0	2.08	2.17	1.3	1.5	136.0	117.2	4.05	25.15
3	0.82	30.9	68.4	1.60	1.60	1.3	1.4	134.3	113.7	5.41	25.05
4	0.83	29.6	63.1	3.19	3.19	1.2	1.5	135.1	112.5	2.77	24.19
5	0.81	19.1	52.1	1.26	1.28	0.9	1.2	136.9	120.9	4.16	24.64
6	0.80	20.0	59.0	1.40	1.39	1.3	1.6	135.4	113.9	3.54	24.35
7	0.82	18.3	54.7	1.03	1.10	1.1	1.4	136.5	117.1	2.54	38.02
8	0.81	23.8	62.7	1.68	1.75	0.7	1.0	137.7	119.2	2.45	37.95
9	0.84	22.4	59.1	1.08	1.06	0.9	0.9	134.1	114.9	2.15	34.31
10	0.81	17.0	56.8	1.08	1.16	0.9	1.2	135.3	116.9	2.44	38.81
11	0.81	27.2	62.1	1.34	1.30	1.0	1.4	133.0	118.6	2.31	35.23
12	0.81	76.5	124.0	2.35	2.30	1.8	1.9	134.6	41.8	2.67	112.60
13	0.82	63.4	99.2	3.07	3.07	2.1	2.4	136.7	119.0	2.82	38.25
14	0.79	62.4	103.2	3.82	3.81	2.1	2.2	138.2	118.3	3.12	37.36
15	0.80	19.6	58.2	1.65	1.76	1.0	1.3	136.5	121.1	2.38	37.03
16	0.86	19.4	54.2	1.87	1.82	0.7	1.0	134.8	119.0	2.42	36.37
17	0.81	29.2	63.2	1.88	1.83	1.0	1.4	136.5	117.7	2.26	40.60
18	0.78	12.2	39.7	1.61	1.61	0.9	1.0	132.8	114.1	2.05	34.39
19	0.81	15.6	53.1	1.22	1.20	1.2	1.3	136.2	112.1	2.33	39.97
20	0.83	28.1	65.1	1.48	1.43	1.0	1.1	135.1	114.0	2.65	37.55
21	0.84	20.2	47.6	1.95	2.02	1.4	1.6	134.4	117.0	2.64	32.86
22	0.81	16.4	52.7	1.23	1.16	1.0	1.4	137.9	116.2	2.16	43.77
23	0.82	20.5	59.5	1.66	1.60	1.4	1.7	138.5	116.7	2.90	42.92
24	0.80	51.9	84.9	2.67	2.81	1.5	1.6	132.0	111.9	5.42	42.56
25	0.80	38.1	76.5	1.71	1.73	0.8	1.0	140.1	120.2	2.38	37.86
26	0.81	27.1	70.6	1.73	1.72	1.4	1.5	137.3	113.0	2.43	41.38
27	0.86	32.0	67.0	2.09	2.35	0.9	1.1	133.1	115.1	2.37	35.85
28	0.83	52.0	88.0	2.49	2.46	1.4	1.6	139.0	123.4	2.37	34.87
29	0.80	32.0	69.0	1.06	1.17	1.4	1.5	135.7	115.9	2.42	34.81
30	0.80	14.0	48.0	0.57	0.62	0.3	0.3	134.2	113.5	2.08	33.68

*Prportion of dialysate effluent fluid on cross-linked polyelectrolyte network

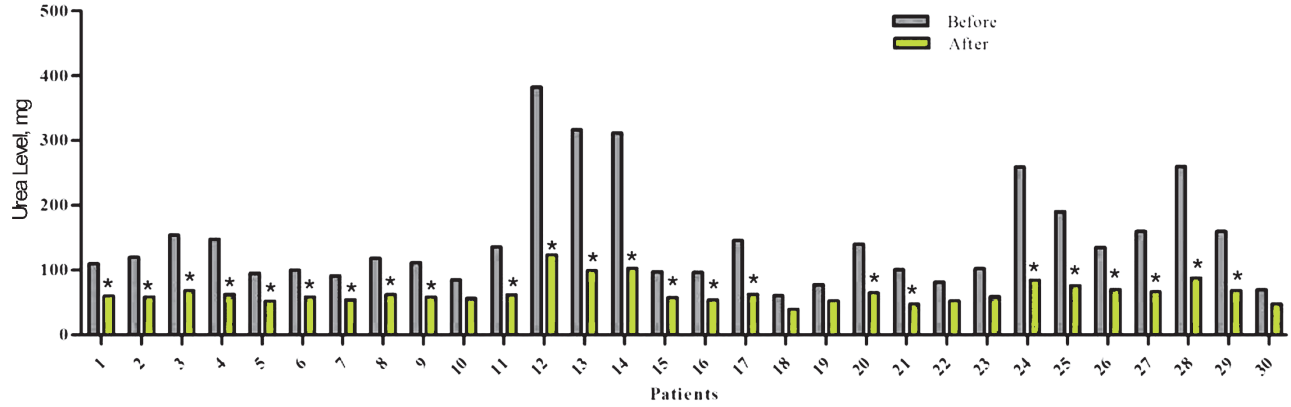

Figure 1. The amount of urea in dialysate effluent samples, before and after treatment with cross-linked polyelectrolyte.

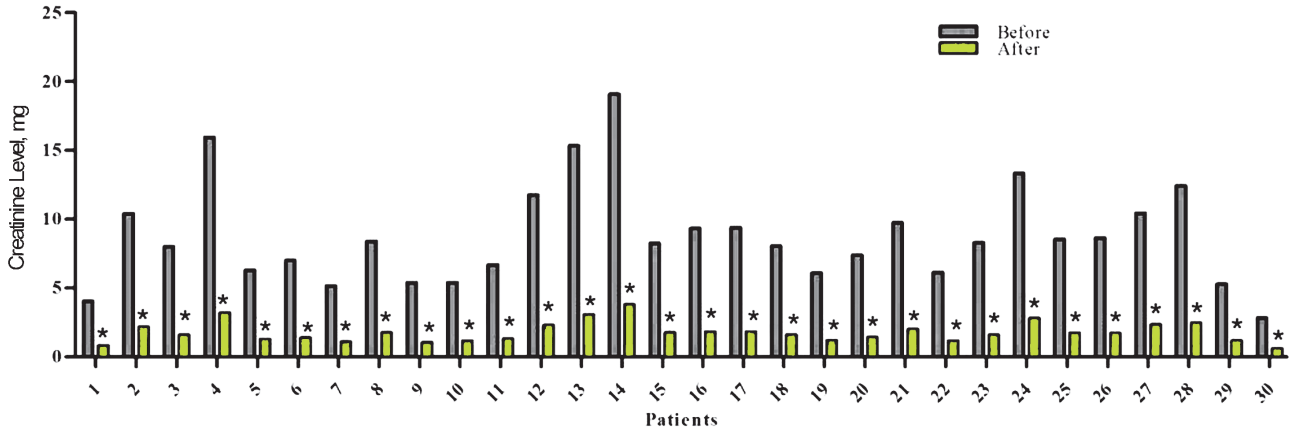

Figure 2. The amount of creatinine in dialysate effluent samples, before and after treatment with cross-linked polyelectrolyte.

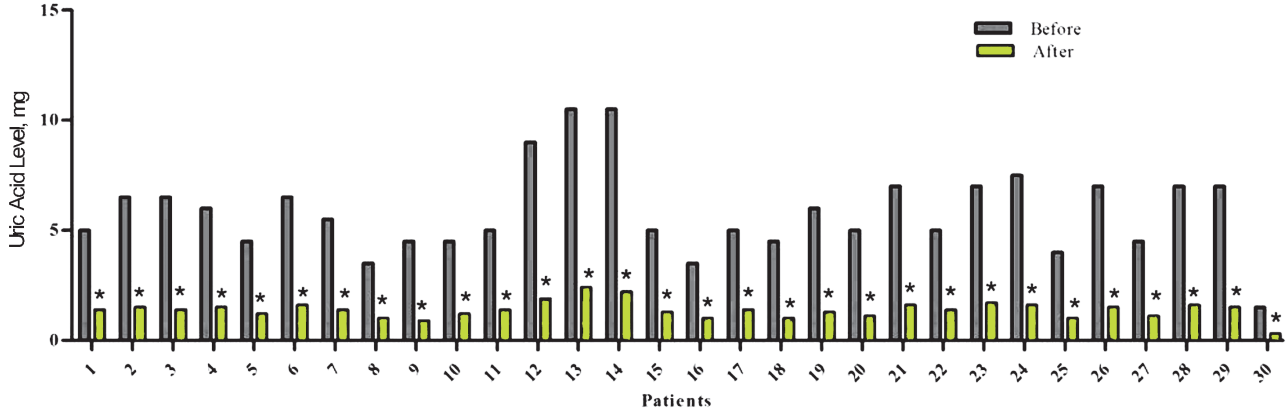

Figure 3. The amount of uric acid in dialysate effluent samples, before and after treatment with cross-linked polyelectrolyte.

DISCUSSION

The dialysis treatment is time-intensive process and the cost of hemodialysis therapy is approximately $ 40 000 to $ 80 000 per year.[21] This has created a significant financial burden on health care systems and the patients. Based on these observations, an improved replacement modality is needed to push back the limitations of the traditional methods. Since several years ago,[15] there has been a trend towards using the bowel as a possible kidney substitute. Unfortunately, in the past decades, this methodology has been overlooked in treatment

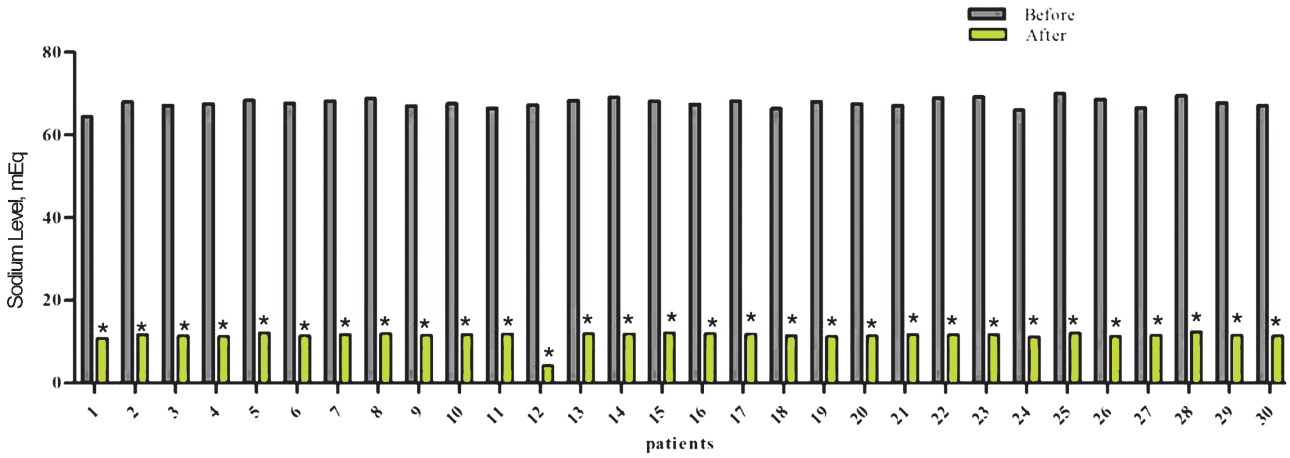

Figure 4. The amount of sodium in dialysate effluent samples, before and after treatment with cross-linked polyelectrolyte.

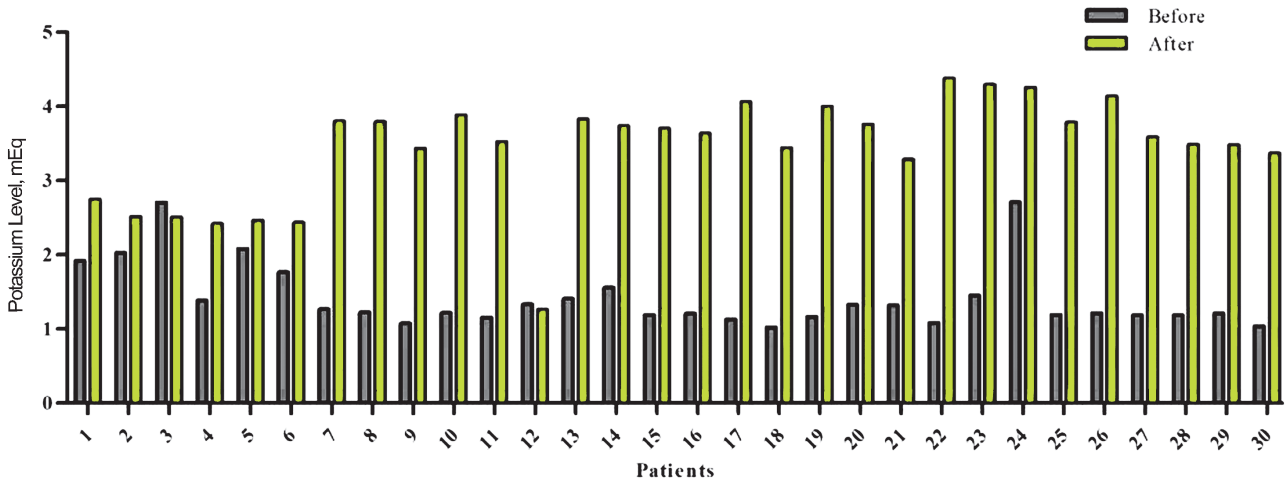

Figure 5. The amount of potassium in dialysate effluent samples, before and after treatment with cross-linked polyelectrolyte.

of CKD patients by developing different dialysis modalities. However, in the past 10 years, there has been a renewed interest in treatment of CKD patients by using the bowel because traditional dialysis modalities are invasive, suffer from high treatment costs, and are associated with high rates of morbidity and mortality.

Bowel fluid contains high levels of urea, creatinine, uric acid and electrolytes, which makes it a potential candidate for extraction of nitrogen wastes. Intestinal fluids in a uremic patient daily contain 2.9 g of creatinine, 70 g of urea, 2.5 g of uric acid, and 2 g of phosphate.[22] These are 5 times more than in normal intestinal solutes. Several different procedures have been used for this purpose, including oral sorbents (eg, AST 120 and chitosan).[23,24] acacia gum,[25] prebiotics and probiotics,[26] diarrhea therapy, and bacterial-enzyme nitrogen recycling within the gut.[27,28] Despite good performance, these methods suffer several limitations, particularly insufficient adsorbing rate of fluid and waste product and electrolytes. As a result, none of those can remove the body excess fluid from the intestinal tract in patients with volume and waste product overload.

In the recent years, CLP has been introduced as new potential material to push back the limitations of current methodologies and achieved efficient extraction of the body excess fluid and wastes products including nitrogenous products from bowel. To date, few studies have examined the therapeutic efficacy of CLP-based treatment in patients with CKD. However, none of these studies, elucidate the detailed events involved in this process.

As it is shown in Table 1, except for patient

3, the level of potassium increased in effluent samples after adding. This finding is in line with previous in vivo experiments presenting that serum potassium concentrations is not affected significantly by CLP. Costanzo and colleagues[15] could not explain the reason for the lack of effect of CLP on serum potassium concentrations. They predicted that changes in the structure of the CLP would be necessary in order to overcome this issue. However, to date, no study has been done with the goal of solving this problem. To our knowledge, this is the first in vitro study to evaluate the abilities and limitations of CLP to remove nitrogen waste products and electrolytes. These results can be related to the types of salts (sodium hydroxide and potassium hydroxide), which are used in the production process of CLPs. Since, in our study and previous work, potassium hydroxide has been used for neutralization of acrylic acid, it seems that nonreduction of potassium can be attributed to potassium hydroxide salt that used in our polymer.

Polyacrylate interaction with metal ions is strongly dependent on ion charge and its chemical activity.[29] The hydrogel which has been used in the present study is partially neutralized by potassium hydroxide. According to data, in the presence of CLP, concentration of potassium ion is increased in most of the dialysate effluent fluid where sodium concentration is decreased. It seems, an ion exchange has been formed between dialysate effluent fluid and potassium containing CLP. Potassium and sodium have equal charge and similar activity but in some cases, they show different manner. According to soft-soft hard-hard theory, potassium ion prefers to interact with soft anions such as sulphur and bromide wherein sodium ion tend to interact with hard anions such as oxygen containing anions.[30] This phenomenon arises from the size and charge of ion. When the size of cation and anion are similar, their interaction will be stronger compared with the interaction of cation and anion that have non-similar size. Therefore, when the effluent fluid is treated with potassium containing polyacrylate, due to better interaction and stable structure, sodium ions migrate from the dialysate effluent fluid to hydrogel structure and exchange with potassium ions and consequently potassium concentration increase in the solution.[31] The mechanisms involved in the interactions of organic compounds and hydrogel network are effected by various factors. These factors include hydrophobic interaction of organic molecule with polymer hydrocarbon chain, hydrogen bonds, organic molecule polarity and charge, hydrogel swelling charge, ion strength, hydrogel charge, and electrostatic interactions. Examining the influences of these factors on adsorption of creatinine, uric acid, and urea needs detailed investigation based on experimental and theoretical analysis, which was out of scope of this study. Fortunately, the behavior of these compounds in those treated with polyacrylate hydrogel has been explained in previous studies.

Undoubtedly, different chemical procedures and therefore different interactions between nitrogen waste products and CLP are the main reasons for noticeable change in the levels of creatinine, uric acid, and urea in effluent fluids. The chemical structure of creatinine, uric acid, and urea has been showed in Figure 6.

Polyacrylate hydrogel network has a negative charge and tend to interact with cationic compounds. For example, these kinds of networks stabilize divalent and polyvalent cations better than monovalent cations such as sodium.[29,31,32] According to acidic and basic data, in effluent fluid pH, creatinine is a neutral molecule,[33] and it can interact with polymer network via hydrogen bonds and adsorbed beside water in hydrogel. In the

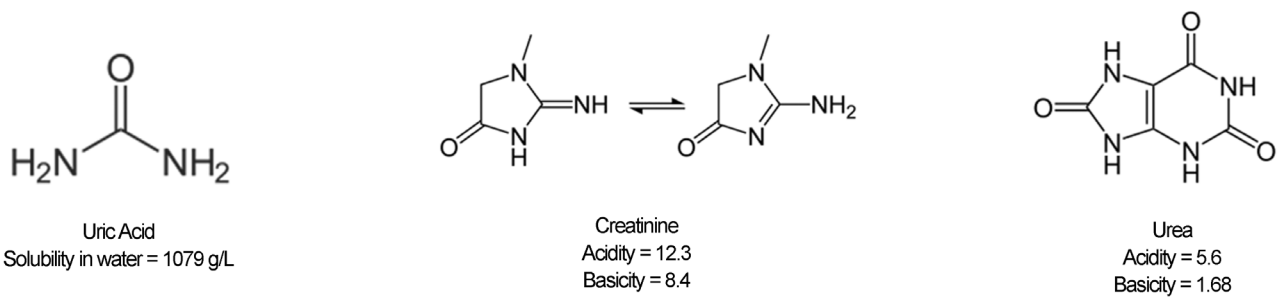

Figure 6. The chemical structure of creatinine, uric acid, and urea.

other words, creatinine has almost same stability in effluent fluid and hydrogel network and therefore its concentration does not change here. Based on these findings, it is evident that the adsorption rate of creatinine and effluent fluid are equal (about 82%). On the other hand, in dialysate effluent fluid pH, uric acid partially dissociates as a weak acid and exists as an anion.[32,34] The electrostatic repulsive forces between uric acid and carboxylate anion force the uric acid to desorb from hydrogel network and its concentration slightly increase in solution. Based on our findings, the adsorption rate of uric acid is about 76%. For instance, 6 g of CLP can adsorb about 4.5 mg uric acid from 500 mL of dialysis effluent fluid containing 6 mg of uric acid.

Finally, the results show significant enhancement of urea concentration in CLP samples. This behavior is laid down in urea special chemical structure. This means that urea does not tend to exist in hydrogel structure. Due to multiple and strong hydrogen bonds, urea can dissolve significantly in water and its solubility is even more than inorganic salts. As a result, urea prefers to be in aqueous media instead of in the hydrogel's ionic media. On the other hand, in the resonance structures of urea, the oxygen atom has a partial negative charge; therefore, the electrostatic repulsive force between urea and negatively charged hydrogel decrease the incorporation of urea in hydrogel structure (Figure 7). Although urea has minimum adsorption percentage on CLP network, it has a maximum adsorption amount on CLP network of 200 mg/g beside water. The adsorption rate of urea is about 50%. For instance, 6 g of CLP can adsorb about 86.1 mg of uric acid from 500 mL of dialysate effluent fluid that containing 154.5 mg of uric acid. Although urea has minimum adsorbing rate among all nitrogen waste products, the amount of adsorbed urea is several times higher than that of uric acid and creatinine.

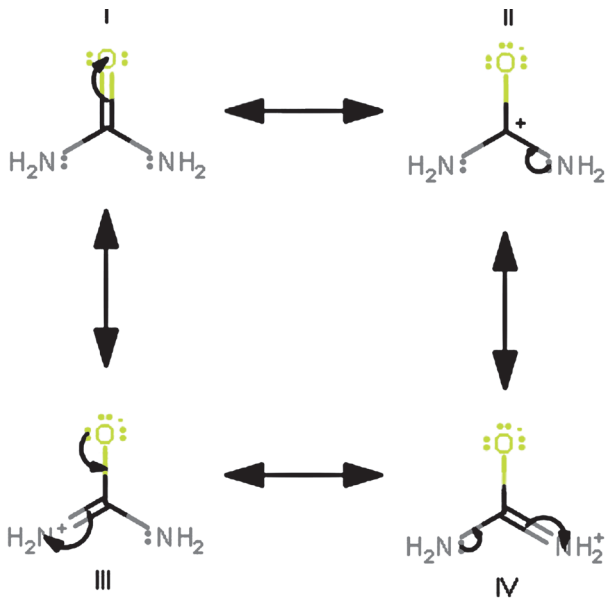

Figure 7. The resonance structures of urea.

CONCLUSIONS

This study introduces a paradigm for using CLP to remove the body excess products from the intestinal tract in patients with volume and waste product overload. However, this work was done in vitro, and other confirming in vivo studies and also preclinical investigations seem to be necessary before its utilization in routine practice. Using CLP in addition to functional medical super adsorbents can be a possible adequate substitute for conventional dialysis methods, especially hemodialysis.

CONFLICT OF INTEREST

None declared.

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