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Evaluation of Some Chemical Parameters of Hemodialysis Water: A Case Study in Iran

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ABSTRACT

BACKGROUND: One of the most common diseases in the world is kidney failure, which can lead to the death of patients. Hemodialysis is a treatment for patients whose kidneys are failing. The water used to perform dialysis must be healthy, safe, and clean. This study aimed to investigate the concentration of heavy metals in hemodialysis water in one of the Hospitals in Iran and compare it with European Pharmacopeia (EPH) and Association for the Advancement of Medical Instrumentation (AAMI) standards.

METHODS: The present study is a descriptive-analytical study conducted on the inlet water of hemodialysis machines in hospital. The samples were collected for 3 months from June to September 2021, Which was examined in terms of free residual chlorine, electrical conductivity, pH, and calcium, magnesium, sodium, aluminum, zinc, copper, and lead concentration. Metals concentration in hemodialysis water was measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technique.

RESULTS: The average value of parameters such as electrical conductivity, pH, residual free chlorine, sodium, calcium, magnesium, zinc, copper and lead in the hemodialysis water was less than the AAMI and EPH standards limits. There was a significant difference at the 95% confidence level with the standard limits, but the aluminum concentration was higher than the standard limits. Also, by examining the medical files of dialysis patients, the most observed problems were anemia and bone diseases, which are probably caused by exposure to high concentrations of aluminum in hemodialysis water.

CONCLUSION: In present study the aluminum concentration is higher than the standard limits. Considering that the higher aluminum concentration can cause diseases such as anemia, bone diseases, nervous deterioration, and death in hemodialysis patients, therefore, it is recommended to continuously evaluate and monitor the quality of hemodialysis water and the performance of its treatment system.

KEYWORDS: Heavy metals, hemodialysis water, hospital, water treatment

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Introduction

Kidney failure is a substantial health concerns in the world. It disrupts blood purification, especially detoxification.[1] Acute kidney injury is still associated with high mortality rate in the world today.[2] Hemodialysis is a treatment for this kind of patients. Hemodialysis removes toxins from the blood and modifies the exchange of water, electrolytes, and chemicals in the blood.[3]

Dialysis fluid is the largest volume of water used in medicine, 99% of which is water purified by the reverse osmosis system.[4] Dialysis patients are exposed to 120 L of water in each dialysis session. On average, about 300 L of water are used per week for dialysis of these patients, which is 30 times more than healthy people. In case of hemodialysis water contamination, these contaminations directly enter the patients' blood.[5]

The dialysis water treatment system consists of deep sand filtration units to remove particulate matter, resin hardener to remove water hardness, activated carbon to remove chlorine and chloramines and reverse osmosis membrane to remove soluble minerals, ions, bacteria, endotoxin, microcystins and heavy metals,[3] that the RO system is able to reduce this parameters, by up to 99%.[6]

In the 1960s, dialysis fluid contamination was discovered and it was found that chemical and microbial contaminants in the dialysis fluid could pass through the dialysis membrane and cause harm to dialysis patients.[7] The presence of chemical and microbial agents in hemodialysis water can have serious and fatal consequences for patients. Dialysis patients often have other chronic conditions, such as cardiovascular disease, hypertension, and diabetes, which make them more vulnerable to adverse outcomes.[8]

Research in different countries has shown that the level of contamination in a significant proportion of dialysis samples was higher than AAMI standard.[9] Raad Humudat et al[10] conducted a study about the estimation of heavy metals concentration in dialysis fluid and blood samples in dialysis patients in Baghdad, Iraq. Their results showed that the quality of the dialysis fluid was not in accordance with the international standards and about 63% of the samples had aluminum concentration higher than the standard. Other studies conducted by Humudat et al in Baghdad hospitals in 4 seasons of the year on the quality of dialysis water revealed that changes in the chemical and microbial quality of water used for dialysis of patients are related to different seasons of the year and a high level of microbial contamination and high concentration of free residual chlorine, calcium, magnesium, aluminum and electrical conductivity in dialysis water. The reason for this issue is the high hardness of drinking water, the aging of the reverse osmosis membrane and the use of aluminum sulfate to reduce the turbidity of drinking water in water treatment plant.[10-12] In another study conducted by Suzuki et al[13] on the quality of dialysis water in Brazil, it was found that the concentration of copper, nitrate and aluminum in dialysis water is higher than the standard limits. The studies of Abualhasan et al[7] in Palestine, Vorbeck-Meister et al[14] in Vienna, Austria, Hilinski et al[15] in Brazil, Ocunola and Olaitan[16] in Nigeria and Jesus et al[17] in Riode Jeneiro, Brezil also showed that the quality of water used for hemodialysis patients was not up to standard.

The amount of trace elements in dialysis fluid significantly disturb the amount of trace elements in the body of dialysis patients.[18] In patients with chronic kidney disease, the cumulative effect of toxic elements remains throughout the disease and exacerbates the complications of the disease.[19] The presence of nitrate in dialysis water causes anemia and lowers blood pressure and the presence of calcium, magnesium and sodium causes high blood pressure, muscle weakness, nausea, and vomiting, neurological disorders and headaches and low pH causes diabetes and nausea and vomiting.[20] In addition to causing acute poisoning in dialysis patients, aluminum also causes bone marrow disease due to interference with the calcium-phosphate balance.[21] Aluminum accumulation is also associated with dementia syndrome and anemia, encephalopathy and osteodystrophy.[21-23]

The presence of zinc and copper in concentrations above the allowable level causes acute poisoning that is associated with anemia, hemolysis, leukocytosis, metabolic acidosis and gastrointestinal symptoms such as nausea and vomiting.[24,25] Lead in high concentrations (12-65 μg/L) causes abdominal pain and muscle weakness in dialysis patients.[26] In a study conducted by Shahryari et al[5] on dialysis water in Isfahan hospitals, the results showed that the concentration of lead, aluminum, calcium, nitrate, and cadmium in the hemodialysis water is higher than the AAMI standard. The reason for this is the insufficient performance of the hemodialysis water treatment system, wear and tear of the water piping system, and the inappropriate quality of the water used in the hospital. The results of this research indicated that although the reverse osmosis system was able to reduce the concentration of many elements to the standard level, it was not efficient enough in removing some others. Table 1 states the complications of hemodialysis water pollution.

Table 1. Complications of dialysis water pollution.[27]

NO.	SYMPTOMS	POTENTIAL WATER POLLUTANTS
1	Anemia	Aluminum, chloramine, copper, zinc
2	Bone diseases	Aluminum, fluoride
3	Hemolysis	Copper, nitrate, chloramine
4	Hypertension	Calcium, sodium
5	Hypotension	Bacteria, endotoxins, nitrates
6	Metabolic acidosis	Low pH, sulfates
7	Muscle weakness	Calcium, magnesium
8	Nervous deterioration	Aluminum
9	Nausea and vomiting	Low pH, bacteria, calcium, copper, endotoxin, magnesium, nitrate, sulfate, zinc, microcystin
10	Visual impairment	Microcystin
11	Liver failure	Microcystin
12	Death	Aluminum, fluoride, endotoxin, bacteria, chloramine, microcystin

All dialysis centers must use strict protocols for water treatment, and water quality must be analyzed periodically. However, water contamination with heavy metals and microbial agents may occur due to the use of unhealthy water sources, problems in the water treatment and distribution system, or the maintenance and disinfection of dialysis machines.[13]

Considering the importance of maintaining the health of kidney patients against complications caused by the inappropriate quality of hemodialysis water, this study aims to determine the quality of hemodialysis water, especially the concentration of heavy metals, and then compare the results with the international standards of AAMI and EPH in one of Iran's hospitals in the Shahreza city was conducted as a case study.

Methods

Study area

The current research is a descriptive-analytical study conducted cross-sectionally on the hemodialysis water of Shahreza Hospital. The studied hospital is located in Shahreza city in the south of Isfahan province (Figure 1). This hospital is

Figure 1. Geographical location of Shahreza in Iran.

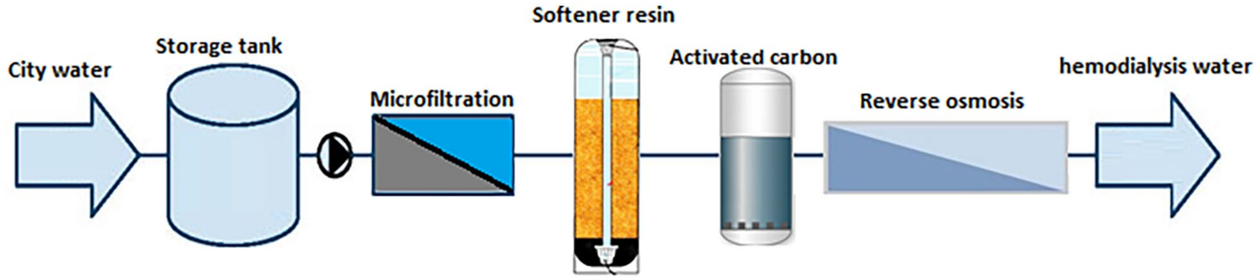

Figure 2. Advanced water treatment system for hemodialysis unit of Shahreza Hospital.

a 250-bed general hospital that has various departments including the Internal Medicine department, General surgery, Operating Room, General Emergency, Gynecology and Obstetrics, Intensive Care Unit, Newborns Intensive Care Unit, Pediatrics, Cardiology, Diagnostic Imaging, Laboratory, General and Specialized clinic, General Services and Dialysis. The dialysis department of this hospital was opened in 2012 with 21 beds, and now 77 patients are undergoing hemodialysis. The source of water used in the hospital is city potable water pipeline, which needs advanced treatment for hemodialysis application. The water treatment unit of this department includes microfiltration, resin hardener, activated carbon, and reverse osmosis membrane (Figure 2). This system supplies 800 L/hour of purified water online for hemodialysis.

Sampling. A total of 12 grab samples were collected from the inlet water of hemodialysis devices from June to September 2021. In present study, parameters of residual free chlorine, pH, electrical conductivity, and concentration of sodium, calcium, magnesium, aluminum, zinc, copper, and lead were measured in hemodialysis water. Falcon polyethylene sampling containers were used for water sampling. At the time of sampling, the water tap was opened, and after about 1 minute after opening the tap, a sample was taken from the current water. The samples to be analyzed should be preserved with concentrated nitric acid (65%) to bring the sample pH down to 2 and the samples were stored in the refrigerator at 2°C. All stages of work from sampling, preservation and analysis were carried out according to the book entitled Standard Methods for the Examination of Water and Wastewater, edition 23, printed in 2017.[28]

Measuring tools. Residual chlorine, pH, and electrical conductivity were measured in situ immediately. For measuring pH and residual chlorine, the Pool Tester kit purchased from Germany Lovibond was applied. Electrical conductivity was measured by EC meter (LF90) manufactured by Germany WWT and concentration of sodium, calcium, magnesium, zinc, copper, aluminum and lead by ICP mass (ajilent7900) which made in Australia. The detection limit was 0.4 µg/L for sodium and calcium, 0.1 µg/L for magnesium and 2 µg/L for lead, aluminum, zinc, and copper, respectively.

Statistical analysis. Data analysis was done with SPSS v26 software. Based on statistical formula (equation (1)) for determining the mean value of parameters, the number of samples was determined equal to 12 at a 95% confidence level.

$$n = \frac{Z_{1-\alpha}{}^{2} S^{2}}{d^{2}} \qquad Eq.1$$

Where Z, S, and d stand for statistic for a level of confidence. (For the level of confidence of 95%, which is conventional, Z value is 1.96), estimated standard deviation from previous studies[29,30] and precision (d is considered about 0.05).[31]

The Kolmogorov-Smirnov normality test were applied to examine if data are normally distributed. The descriptive report of parametric variables was presented as mean and standard deviation and non-parametric variables as median and quartile

Table 2. The concentration of parameters in the hemodialysis water.

VARIABLE	UNIT	MEAN ± SD	MEDIAN	MAX	MIN	STANDARD		*P*-VALUE
						AAMI[32]	EPH[33]	
pH	–	6.74 ± 0.05	6.7	6.8	6.6	–	–	–
EC	μs/cm	24.75 ± 4.2	24.5	32	20	–[a]	–	–
Cl^-	mg/L	0	0	0	0	0.1	–	–
Na	mg/L	4.2 ± 0.8	4	6.1	3.1	70	50	.001*
Ca	mg/L	0.42 ± 0.41	0.3	1.7	0.2	2	2	.008*
Mg	mg/L	0.1 ± 0.3	0.1	0.2	0.1	4	2	.001*

*P-value < .01.
[a]The standard limit according to AAMI, 2004 was 100 μs/cm.

Table 3. The results of measurement of heavy metal concentration in dialysis water.

VARIABLE	UNIT	MEAN ± SD	MEDIAN	MAX	MIN	STANDARD		*P*-VALUE
						AAMI[32]	EPH[33]	
Al	μg/L	14.36 ± 2.54	14.5	27.7	9.6	10	10	.003**
Zn	μg/L	86.5 ± 16.9	90	114.5	54	100	100	.024*
Cu	μg/L	12.3 ± 5.5	12.8	27.6	7.9	100	–	.003**
Pb	μg/L	4.83 ± 1.9	3.5	9.2	3.5	5	–	.143

*P-value < .05. **P-value < .01.

range. Finally, the results were compared with the AAMI and EPH standards. The One-sample *t*-test and the Wilcoxon test were applied to compare normal and non-normal distributed variables with the standard limits.

Investigation of complications caused by hemodialysis water. According to Table 1 (Complications of dialysis water pollution), the file of hemodialysis patients (77 patients) in the studied hospital were carefully evaluated during last 3 years and the most observed problems were reported.

Results

Quantitative physical and chemical analysis of samples is presented in Tables 2 and 3. The mean, standard deviation, median, maximum, minimum, EPH and AAMI standards and the level of significance (*P*-value) of the comparison are given. the residual chlorine was zero in all experiments. The mean and standard deviation of electrical conductivity, sodium, aluminum, and zinc were 24.75 ± 4.2 (μs/cm), 4.2 ± 0.8 (mg/L), 14.36 ± 2.54 (μg/L) and 86.5 ± 16.9 (μg/L), respectively. The median of pH, calcium, magnesium, lead, and copper were 6.7, 0.3 (mg/L), 0.1 (mg/L), 3.5 (μg/L), and 12.8 (μg/L), respectively.

The results of examining complications caused by hemodialysis water in patients showed that out of 77 patients undergoing hemodialysis in the dialysis department of this hospital, 66 people have anemia, 46 people have bone problems, and 1 person has nervous system deterioration.

Discussion

The chemical quality of water used in hemodialysis is quite important. Patients tolerate hemodialysis face serious risks and may even face death if chemical standard limits in hemodialysis water are not managed. These kind of patients are unable to remove toxins from their bodies due to kidney failure. Uremic toxins are defined as substances, organic or inorganic, that accumulate in the body fluids of subjects with acute or chronic kidney disease and impaired kidney function. In addition, the high concentration of toxins in hemodialysis water can lead to excessive accumulation of these elements in the patient's body. This accumulation leads to poisoning and even irreparable consequences for them.[34] So this study aimed to investigate the concentration of some parameters including heavy metals in hemodialysis water and its comparison with AAMI and EPH standard limits.

In normally distributed data, one-sample *t*-test was applied to compare the mean concentration value with the standard limit, and in non-normally distributed data, the Wilcoxon test was used to compare the median concentration value with the standard limit. The one-sample *t*-test explained that the mean concentration of sodium significantly differs from EPH and

AAMI standards and is lower than the standard ($P < .001$). It shows that the dialysis water treatment system has performed well and has been able to reduce the sodium concentration to the standard range in hemodialysis water. A study conducted by Abualhasan et al[7] in Palestine on the water collected from 8 dialysis centers, stated that the concentration of sodium in the hemodialysis water was lower than AAMI standard. The results of this study are consistent with our study.

Since there is no standard for the electrical conductivity of dialysis water mentioned in the AAMI standard of 2019, we compared our results with the standard announced in 2004. The one-sample t-test clarified that the electrical conductivity mean value has a significant difference from AAMI standard limit and is lower than the standard limit ($P < .001$). The obtained results indicate the proper operation of the water treatment system in removing cations and anions from water. The results obtained in study conducted by Zareei et al[35] on hemodialysis centers in Baghdad, Iraq, reported that the electrical conductivity of water was below the AAMI standard limit.

Earlier studies reported that the efficiency of the reverse osmosis system showed different results in various cities worldwide. Investigations by Taleshi et al[36] in Yazd, Asadi et al[30] in Qom, Totaro et al[8] in Italy, Baseri et al[22] in Kashan and Inga Skarupskiene et al[37] in Lithuania revealed that the reverse osmosis system in relation to the elements under study, had 100% efficiency. It was able to reduce the concentration of all elements around the standard limits. However, in studies conducted by Shahryari et al[5] in Isfahan, Pirsaheb et al[21] in Kermanshah, Abualhasan et al[7] in Palestine, Suzuki et al[13] in Brazil and Humudat and Al-Naseri[38] in Iraq, The reverse osmosis system does not have a proper efficiency and has not been able to reduce the concentration of some elements under study, especially heavy metals, to the standard limits. In present study, although the reverse osmosis system was able to reduce the concentration of calcium, magnesium, sodium, zinc, copper and lead under the standard limits, it was unable to reduce aluminum concentration to standard limits.

The one-sample *t*-test showed that the mean concentration of aluminum significantly differs from the AAMI and EPH standards which is higher than the standard limits ($P < .003$). This indicates that the reverse osmosis system was not completely successful in removing aluminum from the hemodialysis water. One of the main sources of exposure to aluminum in hemodialysis patients is hemodialysis fluid.[23] The high concentration of aluminum in the dialysis water can be attributed to the corrosion of the metal pipe system, its release from various synthetic materials such as polyethylene, and then the passage of a part of the aluminum ions through the reverse osmosis system.[19] Other studies are consistent with the results obtained in the present study about aluminum. Studies by Nwobodo et al[39] in South East Nigeria, Shahryari et al[5] in Isfahan hospitals, Sanadgol et al[40] at Khatam Al-Nabi hospital in Zahedan, Vorbeck-Meister et al[14] in Vienna, Austria, Suzuki et al[13] in Brazilian dialysis centers, Humudat and Al-Naseri et al[10] in the dialysis centers of Baghdad, Iraq reported that the mean concentration of aluminum in hemodialysis water was higher than the AAMI standard limit.

Various methods have been proposed to remove and reduce the concentration of aluminum in hemodialysis water. In a case study in England, anion exchange resins were used to remove aluminum in home hemodialysis water, and satisfactory results were reported.[41] Perhaps these resins can be effective as a combined method before and after reverse osmosis to remove aluminum. However, more studies should be conducted. One of the reasons for the high concentration of aluminum in hemodialysis water is the use of aluminum sulfate in water treatment plants.[11] Using coagulants other than aluminum sulfate in water treatment processes can reduce the concentration of aluminum in hemodialysis water. Humudat et al[10,11] suggested the use of 2-stage reverse osmosis system instead of one-stage reverse osmosis to improve the chemical quality of hemodialysis water.

The remaining free chlorine severely damages the reverse osmosis membranes and reduces the reverse osmosis efficiency.[42] As a result, activated carbon filters are used in the hemodialysis section of the water treatment plant. These filters remove chloramines and chlorine from water. In this study, the remaining chlorine concentration in all hemodialysis water samples was zero, which is lower than the AAMI standard limit. Ebrahimi et al[43] studied the microbial quality of water used for hemodialysis in Tabriz city hospital. They stated that the remaining chlorine concentration is zero. This point states that the activated carbon filters in the hemodialysis water treatment systems has been applicable and efficient in chlorine removal from water.

One of the important and effective factors on reverse osmosis efficiency is the temperature and pH of the inlet water. Gedam et al[44] study in India presents the role of these 2 factors in the efficiency of reverse osmosis. Increasing the temperature of raw water reduces the viscosity and increases the speed of raw water passing through reverse osmosis membrane. In this situation the solubility of minerals increases and the rate of solutes diffusion through reverse osmosis also increases. Reverse osmosis removes minerals and thus reduces the pH of water. Acidic pH causes metal pipe corrosion and increases heavy metal concentration in water. The results of our studies showed that the mean pH of hemodialysis water was 6.74 ± 0.05. Due to the fact that there is no standard limit for pH in the AAMI and EPH standards, no comparison was made with the standard limits. But according to the mentioned reasons, it can be understood that maintaining the pH of hemodialysis water in the neutral range can be effective in improving the chemical quality of hemodialysis water.

Regarding the concentration of magnesium, Wilcoxon test demonstrated that the median concentration of magnesium significantly differs from the standard limits and is lower than the AAMI and EPH standards ($P < .001$). Taleshi et al[36] investigation on the hemodialysis water collected from 2 Yazd teaching hospitals and studies on hemodialysis water from 2 canters of Zahedan Hospital by Alizadeh et al[29] indicated that the magnesium concentration in hemodialysis water was lower than the standard limits. Also, Wilcoxon's statistical test revealed that the median concentration of calcium significantly differs from the standard limits and is lower than the AAMI and EPH standards ($P < .008$).

Malaeb et al._ENREF_38,[42] Taheri et al[45] and Lasheen et al[46] studies revealed that various factors such as the life span of hemodialysis water treatment plant, the life span of the water piping system and the quality of inlet water, corrosion of water pipes, inability to managing the dialysis water purification system properly, water pause period In the system, the age and type of pipes, connections of the reverse osmosis purification system and piping can affect the quality of water used in dialysis. In order to improve the quality of hemodialysis water, using a 2-stage reverse osmosis system instead of a one-stage reverse osmosis system can be effective. However, more studies related to environmental issues and reuse of reverse osmosis device effluent should be done to reduce water consumption.[10,11,47]

The one-sample *t*-test revealed that the mean concentration of zinc significantly differs from the standard and is lower than the AAMI and EPH standards ($P < .024$). Studies conducted by Zareei et al[35] in Shahid Beheshti Hospital in Qorveh, Skarupskiene et al[37] in 28 dialysis centers in Lithuania and Al-Naseri et al[48] in Baghdad dialysis centers, discovered that the zinc concentration in hemodialysis water is below the standard range, which is consistent with the present study.

The wilcoxon's statistical test showed that the median concentration of copper significantly differs from the standard limits and is lower than the AAMI standard ($P < .002$). Various investigations on the water used in hemodialysis illustrated that the concentration of copper in the hemodialysis water was lower than the standard limits.[7,22,43,44,48] This indicates that the reverse osmosis system performs well in removing copper from hemodialysis water.

Also, Wilcoxon's statistical test showed that the leads median concentration did not significantly differ from the standard limits and is approximately within the AAMI standard limit ($P < .124$). Investigations carried out by Totaro et al[8] in Brazil, Al-Naseri et al[48] in the dialysis centers of Baghdad, Iraq, Humudat et al[11] in Bghdad, Iraq and Zareei et al[35] in Shahid Beheshti Hospital, Iran, demonstrated that the lead concentration is lower than the AAMI standard.

According to the study conducted in Italy by Tonelli et al,[49] the heavy metals concentration level in patient's blood is correlated with their concentration in hemodialysis water. It means that the concentration of heavy metals in patient's blood increases as the concentration of these metals increases in hemodialysis water. In the investigation of complications in 77 patients undergoing hemodialysis in this hospital, it was observed that 66 patients had anemia (86%), 46 had bone issues (60%), and 1 had nervous system deterioration (1%) which all can be caused by exposure to a high concentration of aluminum.

According to earlier studies as well as the present study, some ions could pass through the reverse osmosis membrane and endanger the health and lives of hemodialysis patients. As a result, it is necessary to continuously or even momentarily monitor the output water quality of the reverse osmosis system. If the concentration of elements and heavy metals are more than standard limits in dialysis water, some interventions such as washing and disinfecting of the filtration system, replacing membranes, replacing pipes and connections and using a 2-stage reverse osmosis system could help to reduce the problem.[11,42,45,46,50]

It should be noted that there were limitations in this study. There was no previous record of heavy metal tests in city water and hospital hemodialysis water. Officials in hospitals should periodically check and monitor the chemical quality of water used in hemodialysis. Additionally, due to the lack of an ICP mass device in study area, we had to preserve and store the samples until the sample analysis. It would have been preferable if the analysis had been completed as soon as possible after sampling.

Conclusion

The present study aimed to investigate the quality of hemodialysis water, especially the concentration of heavy metals, and then compare the results with the international standards of AAMI and EPH. The results of this study stated that the residual chlorine concentration was zero in all experiments, the mean $\pm$SD of electrical conductivity, pH, sodium, magnesium, calcium, aluminum, zinc, copper and lead were 24.75 ± 4.2(μs/cm), 6.74 ± 0.05, 4.216 ± 0.821(mg/L), 0.1 ± 0.3(mg/L), 0.42 ± 0.41(mg/L), 15.5 ± 4.7(μg/L), 86.5 ± 16.9 (μg/L), 12.3 ± 12.8(μg/L), and 4.83 ± 1.9(μg/L), respectively. It could be emphasizing that, the heavy metals can pass through the reverse osmosis system and enter the body of dialysis patients during the hemodialysis process and cause risks for the patients in the long term. It should be noted that the aluminum concentration in hemodialysis water in studied hospital is higher than the standard limits. Based on literatures the higher aluminum concentration can cause diseases such as Anemia, Bone disease, Nervous deterioration and death in hemodialysis patients, therefore, it is recommended to continuously evaluate and monitor the quality of hemodialysis water and the performance of its treatment system. Also, in order to increase the quality of water consumed and reduce the amount of aluminum in hemodialysis water, it is recommended that the hospital authorities put the following actions on the agenda, replacement of filters and membranes on schedule, using of polyethylene pipes instead of galvanized pipes and using of 2-stage reverse osmosis system.

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Authors Contribution

All authors contributed to the design and implementation of this study. All authors performed data analysis and interpretation. All authors wrote, revised and approved the text of the article.

Ethics Approval and Consent to Participate

Ethical approval for this study was obtained from the Ethics Committee of Isfahan university of medical sciences (Ref. no.: IR.MUI.RESEARCH.REC.1400.085). Informed consent was obtained from all individuals and concerned bodies included in this study.

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