



Evaluation of Bacteriological and Chemical Quality of Dialysis Water and Fluid in Isfahan, Central Iran

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Abstract

Background: Chemical and microbial quality of water used in hemodialysis play key roles in a number of dialysis-related complications. In order to avoid the complications and to guarantee safety and health of patients therefore, vigorous control of water quality is essential. The objective of present study was to investigate the chemical and bacteriological characteristics of water used in dialysis centers of five hospitals in Isfahan, central Iran.

Methods: A total of 30 water samples from the input of dialysis purification system and dialysis water were analyzed for chemical parameters. Heterotrophic plate count and endotoxin concentration of drinking water, dialysis water and dialysis fluid of 40 machines were also monitored over a 5-month period in 2011-2012.

Results: Concentration of the determined chemicals (copper, zinc, sulfate, fluoride, chloramines and free chlorine) did not exceed the recommended concentration by the Association for the Advancement of Medical Instrumentation (AAMI) exclude lead, nitrate, aluminum and calcium. Furthermore, the magnesium; cadmium and chromium concentration exceeded the maximum level in some centers. No contamination with heterotrophic bacteria was observed in all samples, while the AMMI standard for endotoxin level in dialysis fluid (<2 EU/ml) was achieved in 95% of samples.

Conclusion: Dialysis water and fluid failed to meet the all chemical and bacteriological requirements for hemodialysis. To minimize the risk of contaminants for hemodialysis patients therefore, a water quality management program including monitoring, maintenance and development of water treatment system in hemodialysis centers is extremely important. In addition, an appropriate disinfection program is needed to guarantee better control of bacterial growth and biofilm formation.

Keywords: Dialysis water, Dialysis fluid, Chemical quality, Endotoxin, Heterotrophic bacteria

Introduction

Patients receiving hemodialysis are exposed to a large volume of dialysis fluid (approximately 120 l) in a single dialysis treatment (1). The presence of a nonselective semipermeable membrane, which acts as a barrier between blood and dialysis fluid, provides a direct route for transformation of contaminants into the blood stream. Consequently, many of permitted levels of chemical substances

in drinking water are potentially dangerous for dialysis patients (2). Some of these chemicals such as calcium, nitrate, sulfate and chloramines cause well-defined acute or chronic poisoning syndromes for these patients (3). High calcium and magnesium levels for instance, cause cardiac problems. Aluminum overload may also lead to encephalopathy, bone disease and anemia in dialysis

patients (4). To minimize patient exposure to potential contaminants of drinking water therefore, additional purification treatment is necessary for water used in dialysis. A series of purification processes such as deionization, carbon filtration and reverse osmosis (RO) are generally used to remove chemical pollutants from water used in hemodialysis. These processes are also an effective barrier against microbiological contaminants (5). In addition, tubing system (hydraulic circuit) of the dialysis machines could promote bacterial growth and biofilm formation. Biofilm acts as a source of bacterial fragments such as DNA and endotoxin which are released into the water and potentially able to penetrate dialysis membranes (1, 6). Endotoxin can induce the production of proinflammatory cytokines. Cytokine induction causes acute symptoms and has been incriminated in the various dialysis-related complications of patients such as dialysis-related amyloidosis, malnutrition inflammation and atherosclerosis syndrome (7, 8). Therefore, the quality of dialysis fluid is a critical factor in the overall care received by dialysis patients and bacteriological quality particularly, has a strong effect on the patient health and outcome (4, 7). To prevent patients from risks of water contaminants a number of standards for quality of dialysis water and fluid have been proposed (2). The Association for the Advancement of Medical Instrumentation (AAMI) standards have represented the most widely standards for the chemical and microbial quality of dialysis water (2, 3). Over the last decade, a number of studies aimed at evaluating the quality of dialysis water and fluid, especially microbial quality, have been performed in developed countries (3, 5, 9). Vorbeck-Meister et al. performed a survey on microbiological and chemical quality of the dialysis water in seven dialysis wards in Vienna, Austria. They observed an increase in endotoxin levels after the water had passed through the tubing system of dialysis machines. They reported that a satisfactory chemical water quality for dialysis could be obtained only by the combination of ion exchange and reverse osmosis (9). A study in 2 dialysis facilities showed viable cell counts of dialysis fluids were less than 10 CFU/ml but colonies were not formed after

passing the endotoxin retentive filters (1). Microbiological results from the central plant and piping ring of 5 dialysis units have led to an improvement in microbiological quality of water subsequent to changes made in the use of materials and procedures. Besides, these changes have a positive impact on the microbiological quality of dialysis patients over a 15-yr period (5).

Based on these premises, the present study was undertaken to investigate the chemical and bacteriological quality of dialysis water and fluid in dialysis centers of five hospitals in Isfahan, Iran. Since, identification of bacteria could be important for assessment of health risks associated with the presence of pathogens and opportunistic bacteria in dialysis fluid (10); the identification of predominant bacteria was also performed.

Materials and Methods

In a cross-sectional study, the bacteriological and chemical quality of the water used in dialysis centers of five hospitals in Isfahan, Iran was analyzed over a 5-month period in 2011-2012. All of centers received water from the municipal drinking water network and used a water purification system consisted of deionization, activated carbon filtration and reverse osmosis.

Chemical analyses

A total of 30 water samples were collected from the input of dialysis water purification system (municipal drinking water) and at the end of purification system after RO (dialysis water). Chemical analyses (concentration of calcium, magnesium, copper, lead, zinc, chromium, cadmium, aluminum, nitrate, sulfate, fluoride, chloramines and free chlorine) were performed monthly on the water samples over a period of 3 months. Residual free chlorine (RC Meter, RC-24P) was determined at the time of sample collection. The concentration of nitrate, chloramines, sulfate, calcium, magnesium, aluminum and fluoride was assayed by DR5000 (Hach Company, USA). Iron, zinc, cadmium, chromium and lead concentrations were measured by flame atomic absorption spectropho-

tometry (Perkin-Elmer 2380). All chemical tests were performed according to the drinking water analyses recommended by *Standard Methods* (11).

Bacteriological analyses

Bacteriological quality (heterotrophic plate count and endotoxin concentration) of drinking water, dialysis water and dialysis fluid of 40 machines in five centers were monitored. The sample port was disinfected with alcohol and allowed to run for 2 min before sampling. Samples were transferred to the laboratory in an insulated box with cooling packs and were processed immediately after arrival at the laboratory.

For heterotrophic plate count, water samples were taken in sterilized 100 ml glass bottles. Fifty ml of water samples were concentrated by centrifugation and aliquots of concentrated and non-concentrated samples were spread plated on R2A agar medium and incubated at 35 °C for 3-5 days. Following incubation, colonies were counted on each plate and results were expressed as colony-forming units per milliliter (CFU/ml) (11). All the experiments were carried out in duplicates and the mean values were considered. Bacterial colonies were also characterized based on the colony and cell morphology on the agar plates and Gram-staining.

Ten ml of each sample was also taken into a sterile pyrogen free tube for endotoxin analysis. Samples were then stored at -25 °C. Endotoxin test was carried out using *Limulus amoebocyte lysate* (LAL) by the gel-clot method (Sigma-Aldrich). The endotoxin (ET) levels for the positive samples were being made semi-quantitative by dilution of the samples with endotoxin free water.

Molecular identification of predominant bacteria

Predominant bacteria of dialysis fluid were isolated and sub-cultured onto R2A agar plates based on their Gram-stain and colony morphology. The isolated colonies were suspended in 100 µl of de-ionized water, and genomic DNA was extracted by boiling for 15 min and centrifugation at 13,000 rpm for 5 min. The supernatant was used for PCR amplification with Eubac 27F and 1492 R primers,

which amplify a ~1,420 bp fragment of the 16s rRNA gene (12). DNA sequencing of the amplified gene was performed, and DNA sequences analysis was undertaken by BLAST algorithms and databases from the National Center for Biotechnology Information (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Statistical analysis

Statistical analysis was performed with SPSS 20.0 (Chigoe, IL, USA). Significant difference between the analyzed parameters in raw drinking water and dialysis water and also with standard values was tested using *t*-test. A *P*-value of <0.05 was considered significant.

Results

The results of chemical analyses of dialysis waters are presented in Table 1. Statistical analysis showed a significantly higher concentration of lead, nitrate, aluminum and calcium in most dialysis water samples than recommended concentration by AAMI. Furthermore, the magnesium; cadmium and chromium concentration exceeded the maximum level suggested by AAMI in some centers. However, these differences were not significant except for magnesium in one center.

Table 2 shows the results of bacteriological analysis of dialysis fluid of 40 machines in five centers. The results indicated that the bacterial count in all dialysis fluid samples was lower than the stated AMMI limits for HPC (<200 CFU/ml) (2). The statistical analysis showed no significant difference between HPC number of raw water, dialysis water and dialysis fluid. The endotoxin analysis revealed that the ET values in all water samples and dialysis fluid were in the limit of guideline values of the AAMI (<2 EU/ml) with the exception of two dialysis machines.

According to the 16s rRNA gene sequence analysis of predominant bacteria, six species of bacteria were identified in dialysis fluid. Species of identified bacteria and accession number for each 16s rRNA gene sequenced are indicated in Table 3.

Table 1: Mean values of chemical parameters of municipal drinking water and dialysis water from the hemodialysis centers of five hospitals, in comparison to Association for the Advancement of Medical Instrumentation (AAMI) standards

| Parameter (mg/L) | AAMI standards (mg/L) | Hospital No. 1 | | Hospital No. 2 | | Hospital No. 3 | | Hospital No. 4 | | Hospital No. 5 | |
|------------------|-----------------------|----------------|-------|----------------|-------|----------------|-------|----------------|--------|----------------|--------|
| | | MW | DW | MW | DW | MW | DW | MW | DW | MW | DW |
| Free chlorine | 0.5 | 0.12 | 0.05 | 0.12 | 0.05 | 0.16 | 0.05* | 0.13 | 0.05 | 0.17 | 0.03* |
| chloramines | 0.1 | 0.07 | 0.00* | 0.07 | 0.01* | 0.06 | 0.02 | 0.04 | 0.01 | 0.05 | 0.03 |
| Calcium | 2 | 57 | 6.75* | 54 | 9.5* | 62 | 19.3* | 30 | 13.9* | 55 | 13.2* |
| Magnesium | 4 | 18 | 2.2* | 17 | 3* | 20 | 6.2* | 9.54 | 4.45* | 17.6 | 4.2* |
| Sulfate | 100 | 63 | 48* | 49 | 32* | 50 | 33* | 42 | 32* | 41 | 14* |
| copper | 0.1 | 0.036 | 0.012 | 0.009 | 0.005 | 0.013 | 0.021 | 0.004 | 0.008 | 0.009 | 0.007 |
| Zinc | 0.1 | 0.028 | 0.02 | 0.02 | 0.007 | 0.015 | 0.04 | 0.06 | 0.11 | 0.036 | 0.006* |
| Cadmium | 0.001 | 0.001 | 0.001 | 0.006 | 0.002 | 0.012 | 0.006 | 0.016 | 0.002* | 0.013 | 0.001* |
| Chromium | 0.14 | 0.004 | 0.011 | 0.01 | 0.014 | 0.017 | 0.016 | 0.026 | 0.017 | 0.021 | 0.008 |
| Lead | 0.005 | 0.026 | 0.016 | 0.03 | 0.024 | 0.026 | 0.031 | 0.028 | 0.034 | 0.031 | 0.0138 |
| Aluminum | 0.01 | 0.1 | 0.08 | 0.1 | 0.09 | 0.06 | 0.04 | 0.06 | 0.06 | 0.04 | 0.03 |
| Nitrate (as N) | 2 | 7.7 | 5.3* | 6.1 | 4.4* | 6.8 | 2.8* | 5.8 | 2.4* | 5.8 | 3.2* |

MW: Municipal drinking water (input water).

DW: Dialysis water

* There was a significant difference between the concentration of analyzed chemical parameter in MW and DW (P -value <0.05).

Table 2: Heterotrophic plate count (HPC) level and endotoxin concentration of dialysis fluid in five dialysis centers

| Hospital No. | Number of samples | Percent (%) of samples with endotoxin concentration* | | | % of samples with HPC** | | |
|--------------|-------------------|--|-------------|-----------|-------------------------|---------------|-------------|
| | | <0.5 EU/ml | 0.5-2 EU/ml | > 2 EU/ml | <50 CFU/ml | 50-200 CFU/ml | >200 CFU/ml |
| 1 | 4 | 100 | 0 | 0 | 100 | 0 | 0 |
| 2 | 6 | 50 | 50 | 0 | 100 | 0 | 0 |
| 3 | 10 | 80 | 10 | 10 | 100 | 0 | 0 |
| 4 | 8 | 62.5 | 25 | 12.5 | 100 | 0 | 0 |
| 5 | 12 | 92 | 8 | 0 | 100 | 0 | 0 |
| Total | 40 | 77.5 | 17.5 | 5 | 100 | 0 | 0 |

*AAMI standard for endotoxin: <2 EU/ml

**AAMI standard for HPC: <200 CFU/ml heterotrophic plate count

Table 3: Predominant bacteria identified by 16S rDNA sequence analysis

| Bacterial species | Accession number |
|-----------------------------------|------------------|
| <i>Pelomonas saccharophila</i> | KM262798 |
| <i>Sphingomonas adhaesiva</i> | KM262799 |
| <i>Bacillus subtilis</i> | KM262800 |
| <i>Dechloromonas agitata</i> | KM262801 |
| <i>Bacillus licheniformis</i> | KM262802 |
| <i>Porphyrobacter donghaensis</i> | Km262803 |

Discussion

Since, the quality of dialysis fluid plays an important role in patient safety and welfare; it should be viewed as a medicinal product and every effort should be made to ensure a high quality fluid (5). Water purification system in hemodialysis centers especially, reverse osmosis leads to a sufficient decrease in the amount of contaminant parameters. The results of our study showed that the chemical quality of drinking water is not acceptable as dialysis water because of the presence of some chemicals in higher concentrations than recommended by standards for dialysis water. Based on the results, concentration of lead, nitrate, aluminum and calcium in drinking water exceeded maximum levels suggested by the AAMI and the purification systems couldn't significantly reduce these chemicals in all centers. In other words, the chemical quality of water coming out of the RO in all centers was not completely suitable as the dialysis water (Table 1). Furthermore, concentration of magnesium; cadmium and chromium of dialysis water in some centers didn't comply with the AAMI guidelines (Table 1). These results indicate that water treatment by purification systems in some centers couldn't lead to a sufficient decrease in concentration of these chemicals. Nitrate, calcium and magnesium may be naturally present in the drinking water which is used. However, some chemicals may originate from procedures applied in the treatment of water or may be released from water pipes (3, 9). Generally, RO-based treatment systems produce dialysis water of optimal chemical quality. However, the efficacy of the systems depends on the maintenance and operation (3). Based on the chemical analyses, a little higher concentration of zinc, copper and lead were found in dialysis water samples than drinking water from two centers, which may have caused by materials in the treatment systems or chemicals used. Therefore, special attention must be paid to the suitability of materials and chemicals used in dialysis treatment systems (9).

As shown in Table 2 all CFU values for dialysis fluid were lower than the stated AAMI limits for

HPC (<200 CFU/ml). CFU values exceeded the European Pharmacopeia value (<100 CFU/ml) in 12.5% (n=40) of dialysis water samples (9). A study of hemodialysis centers of nine hospitals in Japan showed that the AAMI limit for microbial count was exceeded in one (5.5%) of all 18 water samples and the microbial count of dialysate (dialysis fluid) was significantly higher than of treated water (13). The results of our study showed no significant difference between HPC number of raw water, dialysis water and dialysis fluid. However, it was noticeable that most of dialysis water samples had higher CFU values in comparison to drinking water samples. Our finding confirms that ion exchange resins and activated carbon filtration generally promote bacterial growth (3, 5). Therefore a UV disinfection step in the dialysis treatment system is favorable from the microbiological and safety point of view (9).

Endotoxin concentration in dialysis water of all centers was in the limit of guideline value of the AAMI (<2 EU/ml). However, endotoxin concentration exceeded the limit value after the water had passed through the tubing system of the two dialysis machines in hospital centers 3 and 4 whereas, CFU values were below the standard limit (Table 2). The result is in accordance with other studies that found discrepancies between the levels of ET and bacterial count (1, 7, 14) in dialysis fluid. For this reason, to evaluate the bacteriological quality of dialysis fluid, both ET concentration and viable cell count should be measured (1, 14). Collection of the data from 3488 dialysis facilities in Japan showed that the Japanese Society for Dialysis Therapy (JSDT) standard for the bacterial cell count (<100 CFU/ml) and ET level (<0.050 EU/ml) in dialysis fluid was achieved in 96.9% and 89.0% of facilities (7). In the study of Lima on microbiological quality of water from hemodialysis services in Sao Luis, Brazil; heterotrophic bacteria and endotoxin contamination were detected in 66.6% and 33.3% of the post-treatment samples, respectively (15).

To reduce the risk of endotoxin contamination, periodic cleaning and disinfection of the dialysis machines are essential (9). Although, all centers used daily chemical disinfection procedure of the

dialysis machines in the morning; we found endotoxin values exceeding the guideline value of the AAMI for the two dialysis machines. Double ultrafiltration of dialysis fluid resulted in sterile fluids with endotoxin concentration well below the European Pharmacopeia standards (16). Application of ultrapure dialysis fluid is also associated with a range of clinical benefits (2).

According to the 16s rDNA sequence analysis of predominant bacteria in dialysis fluid, two and four species of gram positive and gram negative rod shaped bacteria were identified, respectively (Table 3). *Bacillus Subtilis* and *B. licheniformis* are gram positive, rod-shaped bacteria. *Bacillus* spp. commonly isolated from water distribution systems (17). These bacteria produce spores that are quite resistant to disinfection (18) and therefore could be found in dialysis water and fluid as reported in other hemodialysis studies (10, 19, 20). *Sphingomonas adhaesiva* and *Pelomonas saccharophila* are gram negative, rod-shaped, non-spore forming bacteria. Gomila et al. described two other species of the *Pelomonas* in dialysis fluid (21). They also described the role of *Sphingomonas* in the build-up of biofilm in hemodialysis systems (10) and *S. paucimobilis* detected by Oie et al. in dialysate samples (13). *Sphingomonas* spp are a group of chemoheterotrophic strictly aerobic rod-shaped bacteria that are widely distributed in the nature and found in water distribution lines (18). *Porphyrobacter donghaensis* and *Dechloromonas agitate* are also gram negative bacteria and presence of *P. donghaensis* in sea water was reported (22).

Conclusion

The dialysis water in all centers failed to meet the all chemical requirements for hemodialysis. Failure to ensure adequate water chemical quality may have dire consequences to patient safety and welfare. Therefore, dialysis water must be monitored routinely and a constant and vigorous control of hemodialysis water treatment system is essential. Furthermore, the endotoxin contamination of two dialysis machines indicates the need for an appropriate

disinfection program to guarantee better control of bacterial growth and biofilm formation.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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