ORIGINAL ARTICLE

IMPACT OF THE CHEMICAL DISINFECTION AND DOUBLE REVERSE OSMOSIS ON DIALYSATES: QUALITY AND THE INFLAMMATORY STATUS OF PATIENTS

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Abstract

Background: Renal hemodialysis requires enormous amounts of water for functioning. In order to avoid complications with patients' blood, this water must be treated. The objectives of this study were to investigate chemical and microbial contamination of treated water, to evaluate the disinfection protocol applied and the double reverse osmosis installation, and to determine the effect of double reverse osmosis on the patient's inflammatory status. **Methods**: Our study was done at the Al Ghassani hospital hemodialysis unit's. Samples were taken at the old and new units. The chemical and microbiological analyses were performed according to national standards. Non-compliance was determined by comparing the results with the values reported by national guidelines. The CRP was determined by the Immuno turbudimetrie-test. **Results**: Non conformity caused by nitrates, pH and conductivity was observed both in a few samples before disinfection and introduction of the double reverse osmosis. Treated water and dialysates showed counts of heterotrophic bacteria lower than 100 CFU/ml. We noted differences in contamination level between the first and second period both in starting loop, dialysate and in C-reactive protein level. An improvement in the quality of the treated water was observed after chemical disinfection of the loop in new unit. **Conclusions**: Non-compliance of chemical results was observed both before disinfection and after introduction of the double reverse osmosis. However, Microbial results are in accordance with national standards for dialysis water. The implementation of double reverse osmosis in the new unit showed a highly significant improvement.

Keywords: *Dialysate Fluid Compatibility and Quality, Hemodialysis Delivery Systems, Inflammation, Quality Improvement, Water Treatment.*

1. INTRODUCTION

Renal hemodialysis is a process that allows the survival of a patient with kidney failure. It allows treating patients by blood through a semi permeable membrane filtration against a saline solution, thus eliminating excess electrolytes, some toxic wastes and water [1]. It is the most widely practiced method of supplementing renal insufficiency [2]. In France, of the 73,491 patients with end-stage renal disease reported in 2012, 56% are treated with hemodialysis [3]. In the USA, there were 120,688 newly reported cases of end-stage kidney disease, representing a slight increase of 1.1 percent compared to 2013. At the end of 2014, there were 678,383 dialysis and transplant patients receiving treatment for end-stage kidney disease, up 3.5 percent from 2013 [4]. In Morocco, around 4 000 new cases of patients with End Stage Renal Diseases (ESRD) are being found each year [5]. Kabbali and al. reported in 2012, that 2,067 patients have been treated in 39 hemodialysis units in four regions of Morocco: Fez-Boulemane; Taza-Taounate-Houceima; Meknes-Tafilalet, Oriental [6]. During a hemodialysis session, a patient's blood is exposed 30 times to water more than what ingests an individual not suffering from renal insufficiency [7]. As water makes up more than 97% of the solution and for dilution of hemodialysis concentrates, it is treated and the maximum admissible values of the different compounds found in the treated water are regulated [5]. Several recommendations and standards regarding microbiology and chemistry exist. In all of them, limits for microbiological and physico-chemical quality are given [8].

Contaminated dialysate and water used for dialysate preparations with bacteria such as Gram negative bacilli can cause infections and pyrogenic reactions [9].The increased bacterial load in water increase the risk of a breakthrough of the patient's blood through the semi permeable membrane of the dialyzer [5]. Inadequate disinfection of distribution systems and water pipes inside the dialyzers was implicated in several outbreaks of bacteremia gram

negative and pyrogenia in hemodialysis (HD) units [10]. Consequently, disinfection protocol must be prevented and frequent to assure a control quality of dialysis unit [11].
In the context of improving the treatment quality and the prevention of infectious risk in hemodialysis, and following the previous work carried out [12, 13], we conducted the following study. Its objectives were: (i) to assess the quality of dialysate and water preparation dialysate both in old and new units of Al Ghassani hospital; (ii) to evaluate the disinfection protocol applied and the double reverse osmosis installation; (iii) to determinate the effect of double reverse osmosis on the inflammatory status of patients who are receiving long term HD of ESRD.

2. MATERIALS AND METHODS

2.1 Research site

At both old and new dialysis units of Al Ghassani hospital in Fez Prefecture, located in the north central region of Morocco, we investigated chemical and microbial contamination of treated water (starting and back loop), acid and bicarbonate concentrates and dialysate. The first part of this research was conducted in 2011 (March-June) in the old unit, while the second one on 2014 (May-June) in the new one. The old unit started in March 1996, while the new one was built according to International standards and started in September 2012; it differs from the old unit of its largest capacity and the implementation of the double reverse osmosis. This unit performs approximately 1200 hemodialysis sessions per month for the 94 patients suffering from a chronic kidney disease.

2.2 Samples: 88 samples were taken at the old unit and 46 at the new unit. They were focused on starting loop (n=8), acid concentrates (n=42), bicarbonate concentrates (n=42) and dialysate (n=42). The sample port had been disinfected with alcohol, and flushed for 2–3 min before 500 ml of sample was taken into appropriate bottles. Acid concentrate and bicarbonate concentrate samples (500 ml each) were aseptically obtained from their holding tanks. Dialysate samples were obtained in the same manner as treated water samples directly before dialysis. Both in old and new unit, the first investigations were conducted immediately before chemical decontamination applied to the processing loop while the latest surveys were made immediately after periodic disinfection.
The samples were delivered quickly in an ice maintained at 4°C to the Regional Diagnostic Laboratory Epidemiological and Environmental Hygienic, to be analyzed.

2.3 Chemical analysis: Chemical parameters (pH, electrical conductivity at 25°C, nitrate, nitrite and sulphate) were measured according to the methods described by Rodier and al., (2003) [14]. Non-compliance was determined by comparing the results with the values reported by national guidelines and European Pharmacopoeia [15, 16].

In addition, two water samples obtained from the starting loop and dialysate were used for analysis of heavy metals at the Interface Regional Academic Unit. 14 heavy metals were measured: Aluminium, Arsenic, Beryllium, Cadmium, Cobalt, Crome, Copper, Iron, Lithium, Manganese, Molybdenum, Lead, the vanadium and Zinc. The dosage of these metals was carried out by the ICP (Inductively Coupled Plasma) method.

2.4 Bacterial analysis: The samples used for microbiological testing were collected aseptically in sterile flasks. To estimate the number of heterotrophic plate count bacteria, the membrane filter technique was employed. A volume of 100 ml of the samples was filtered through membrane filters with pores 0.45 mm in diameter (Sartorius®). The membranes were then placed face up on agar yeast extract (Biokar) and incubated for 72±3 h at 37±1°C. The maximum level of total flora (heterotrophic bacteria) is defined by a Moroccan Ministerial Decree [15], who recommends a threshold of less than 100 colony forming units (CFU)/ml. Conventional microbiological methods were used for identification of bacteria isolated from water treatment system and dialysate: Gram, Oxidase, Catalase, Coagulase, motility, oxidation-fermentation (OF). It is important to note that the quality of all tests used control was carried out by reference strains (*E. faecalis* and ATTCC *E .coli* LMG 21085 51299). Besides, equipment used (incubator, refrigerator, thermometer, etc.) are checked daily and controlled internally by quality procedures and are connected by an accredited laboratory.

2.5 C-reactive protein analysis: The Immuno turbudimetrie-test was used for the quantitative determination of C-reactive protein (CRP) in serum and plasma of the patients treated both in 2011 and 2014 (n=64). This assay was performed in the clinical laboratory of the Fez University Hospital Hassan II.

2.5 Statistical Analysis: Statistical analysis was done by EPI-Info version 2003. Results are reported as mean ± standard deviation. The $p < 0.05$ was deemed as statistically significant.

3. RESULTS

3. Chemical analysis

Both in old and new units, nitrites have not been registered in all dialysates. The highest rate of sulfates was 0.82mg/l. Non conformity caused by nitrates, pH and conductivity was observed in a few samples before disinfection. The maximum of pH and nitrate were respectively 8.1 and 2.37 mg/l.

As shown in table 1, the carried disinfection improved pH and rate of nitrate, but the differences weren't significant. Concerning electrical conductivity, we can obviously see that it wasn't conforming even after disinfection in spite of the significant difference.

Table 1: Mean values of dialysate physico-chemical parameters analyzed before and after disinfection.

	Before disinfection	After disinfection	P value
Potential of hydrogen	7.14±0.378	7.00±0.00	0.356
Electrical conductivity (µS/cm)	14.29±0.488	15,00±0.00	0.008
Nitrate (mg/l)	0.0405±0.0312	0.0291±0.0230	0.143
Sulfate (mg/l)	0.71±0.488	1±0.000	0.172

As shown in table 2, the rate of heavy metals analyzed were lower than required values (< 0.01 mg/l) which prove compliance of these parameters with respect to national regulations and European Pharmacopoeia.

Table 2: The table presents the rate of heavy metals.

Code of heavy metals	Rate (mg/l)	Limite value
Al	<0.01	5
As	<0.01	0.1
Be	<0.01	0.1
Cd	<0.01	0.01
Co	<0.01	0.01
Cr	<0.01	1
Cu	<0.01	2
Fe	<0.01	5
Li	<0.01	2.5
Mn	<0.01	0.2
Mo	<0.01	0.01
Ni	<0.01	2
Pb	<0.01	5
V	<0.01	0.1
Zn	<0.01	2

As shown in table 3, the mean values of dialysate physico-chemical parameters analyzed before and after osmosis installation revealed a significant difference for both electrical conductivity and potential hydrogen.

Table 3: Mean values of dialysate physico-chemical parameters analyzed before and after osmosis installation.

	Before reverse osmosis installation	After reverse osmosis installation	P value
Potential of hydrogen	7.728±0.1632	7.30±0.0962	0.005
Electrical conductivity (µS/cm)	12.733±0.2264	14.58±0.1209	0.000

4. Bacterial analysis

Among the samples analyzed, neither the acid nor bicarbonate concentrates showed microbial contamination, in contrast to treated water and dialysates. However, the samples of starting loop and dialysate showed counts of heterotrophic bacteria lower than the limit permitted by national standards for water dialysis: 100 CFU/ml. Table 4 lists the mean values of total bacteria in starting loop and dialysate obtained before and after disinfection protocol.

Table 4: Mean values of total bacteria in starting loop and dialysate obtained before and after disinfection protocol.

	Before disinfection (CFU/100ml)	After disinfection (CFU/100ml)	P value
Starting loop			
In Old unit	621.00±41.012	525.00±173.948	0.493
In new unit	74.00±11.314	6.00±4.243	0.047
Dialysate			
In Old unit	508.43±179.068	453.64±378.820	0.588
In new unit	85.00±97.096	32.43±40.603	0.272

During the study periods, we showed statistically significant difference between treated water analyzed before disinfection and those evaluated after disinfection in new unit (p=0.047) but the difference between the same samples was not significant in old unit (p = 0.493). Monitoring contamination levels showed an increase in the

bacterial count after disinfection in some dialysates. However, the differences weren't significant both in old unit (p=0.588) and the new one (p=0.27).

In all starting loop and dialysate, 101 Bacterial isolates have been found. As shown in table 5, 80.19% of them were isolated from dialysate, 19.81% from starting loop. 78.21% of them were found in old unit while 21.79 were noted in new unit.

Table 5: Frequency (number) of bacteria isolated from starting loop and dialysate samples in the old and new unit.

	Bacteria Isolated	New Unit	Old Unit
Starting loop	*Bacillus sp*	0	0
	Staphylococcus aureus	1	5
	Staphylococcus sp	0	0
	Streptococcus sp	0	1
	Gram-negative bacilli	4	9
Dialysate	*Bacillus sp*	1	7
	Staphylococcus sp	7	12
	Staphylococcus aureus	0	1
	Streptococcus sp	1	1
	Gram-negative bacilli	8	43

In order to appreciate the double reverse osmosis's implementation's effectiveness, we compared on one hand, the average total aerobic flora on the dialysate and starting loop obtained in the old and new unit, and on the other hand, inflammatory status of patients treated also at the old and new unit.

Table 6 shows the mean values of total bacteria in starting loop and dialysate obtained before and after double reverse osmosis installation (expressed in CFU/100ml). We noted statistically significant differences in contamination level between the first and second period both in starting loop (p=0.002) and dialysate (p=0.004).

Table 6: Mean values of total bacteria in starting loop and dialysate obtained before and after reverse osmosis.

	Before reverse osmosis installation	After reverse Osmosis installation	P value
Starting loop	573.00±117.127	40.00 ±39.875	0.002
Dialysate	334.929±128.7894	58.71±47.194	0.004

After double reverse osmosis implementation, the C-reactive protein (CRP) levels of patients treated both in old and new unit decreased. The difference was statically significant (p = 0.007).

4. DISCUSSION

In our hemodialysis unit, 132 patients are receiving long-term HD for the treatment of end-stage renal disease. Water treatment and dialysate are a vital aspect of the practice of safe and effective HD for these patients. Evaluation of physicochemical and microbiological water quality is an essential process in the prevention of infections in hemodialysis.

In this context, we conducted four physico-chemical and microbiological control cycles of the dialysates and starting loop, taken in the hemodialysis units (old and new), located in Al Ghassani hospital. These checks were carried out in a first time before and after disinfection, and before and after implementation of the double reverse osmosis in a second time.

The physico-chemical analysis included pH, electrical conductivity, sulfates, nitrates, nitrites, heavy metals. The rates of nitrite and sulfates were respectively lower than 0.005 mg/l and 50mg/l. The analyzed heavy metals were lower than required values , which proves compliance of these parameters with respect to national guidelines and European Pharmacopoeia [15, 16].

Non-compliance caused by nitrates, pH and conductivity was observed both before disinfection and introduction of the double reverse osmosis, in a few samples. The disinfection carried improved pH value and rate of nitrate, but their differences weren't significant, though it did not improve the levels of electrical conductivity. Whereas the double reverse osmosis improved pH. In fact, an excess of nitrate has been found only in some samples, however it should not be neglected in order to prevent methemo-globinemia that may result [17].

All bacteriological test results were within national guidelines (<UFC/100ml). Nevertheless, among HD patients, clinical studies have confirmed that hemodialysis water, despite acceptable levels of bacterial contamination may cause a pyrogenic reaction and chronic inflammatory state [18, 19]. Microbial contaminants, including fragments of endotoxin, peptidoglycans, and fragments of bacterial deoxyribonucleic acid, can cross both low-flux and high-flux membranes, stimulate cytokine production and trigger elevation of acute phase reaction proteins like C-reactive protein CRP [20].

In this study, neither the acid nor bicarbonate concentrates showed microbial contamination, in on contrary to starting loop and dialysates. In addition, the microbial contamination level of dialysates was higher than that of the starting loop as well after disinfection, as after the introduction of the double reverse osmosis. The contaminant bacteria were not common in treated water and dialysate. These results are in agreement with other authors [9, 21] and suggest that the dialysis machine is the main source of contamination. Tubing within the dialysis machine may be the site of biofilm development, resulting in high level contamination of dialysate Even if dialysis machines are disinfected daily, biofilm contamination may not be completely eradicated [21]. Both, in old and new unit, the disinfection protocol is chemical and performed only 4 times per year, or once a quarter. Furthermore, in old unit, treated water was stored in reservoir holding tank from where it is distributed to dialysis machines. Bommer and al. (1987) had reported that water stagnancy is a contributing factor to bacterial contamination of the water in the pipe systems [22].

Several bacteria were isolated both from the starting loop and the dialysate; we isolated Gram-negative bacilli at high frequency both in old and new unit (62.92% in dialysate, 65% in starting loop). These results are in accordance with other authors [9, 21]. Lima and al. (2005) reported that these bacteria are able to grow rapidly even in sterile water and dialysis fluids [23]. Marion-Ferey and al. (2002) reported that the roughened inner surface of the silicone tube, the optimal temperature of 37°C and the presence of glucose and bicarbonate in the dialysate, provide ideal conditions for the growth of the biofilm [24].

The mean values of total bacteria in starting loop and dialysate obtained before and after double reverse osmosis installation, showed statically significant differences in contamination level between the first and second period both in starting loop (p=0.002) and dialysate (p=0.004). We noted statically significant differences in C-reactive protein level between the first and second period (p=0.007). These results suggest that the use of pure water leads to a level of contamination of the order (0.4CFU/ml) in starting loop and (0.5 CFU/ml) in dialysate. Several studies have shown that the use of ultrapure water defined as microbial contamination of <0.1 CFU/mL and endotoxin contamination of <0.03 IU/mL, leads to a significant decrease in inflammatory parameters [19, 25]. Using of ultrapure dialysis fluid for all patients and all dialysis modalities was recommended worldwide [11, 16].

Despite the significant improvement in the dialysates qualities after the introduction of double reverse osmosis, we propose the coupling of the thermal disinfection daily distribution piping and chemical disinfection biweekly. Alayoud and al. (2014) have demonstrated that the use of a single chemical or thermal disinfection alone is not enough for maintaining a maximum water preparation rate of dialysate purity [26]. These authors were able to isolate microorganisms from the reverse osmosis unit after thermal disinfection, and suggested that the dead space present in the reverse osmosis unit cannot be sufficiently exposed to heat, but accessible chemical disinfection [26]. Furthermore, an ultrafiltration membrane placed immediately before the entrance of dialysate into the dialyzer has been recommended as a measure against the microbial contamination of dialysate [9, 27].

5. CONCLUSION

Non-compliance caused by nitrates, chlorides, pH and conductivity was observed both before disinfection and after introduction of the double reverse osmosis, in some samples. The results of microbial analysis are in accordance with national standards for dialysis water. The implementation double reverse osmosis in the new unit showed a highly significant improvement. However, we isolated several bacteria from the treated water used in the dialysis fluid and in the kidney machine. To reduce the level of microbiological and physico-chemical dialysate contamination, we recommend coupling the chemical disinfection to thermal disinfection, and to place an ultrafiltration membrane immediately before the entrance of dialysate into dialyzer, and piping change in generators in order to fight against biofilm.

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