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Research of endotoxins in hemodialysis water

Pesquisa de endotoxinas em água de hemodiálise

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ABSTRACT

Introduction: Chronic kidney disease is characterized by loss of kidney function leading to a physiological and biological functional imbalance. One of the treatments necessary for the chronic renal patient is hemodialysis. Water quality is critical to reducing patient risk. Resolution RDC n. 11, of March 13th, 2014, from Brazilian Health Regulatory Agency (Anvisa) establishesd the search for endotoxins, as venous contamination by endotoxins may cause various problems in the patients' health. **Objective:** To identify endotoxins in purified water for hemodialysis. **Method:** This is a Quantitative Descriptive Cross-sectional Study to verify the presence of endotoxins in 126 water samples collected at two points (post-reverse osmosis and reuse room) at six renal substitution therapy clinics, by the State Sanitary Surveillance, and analyzed in the LACEN, in Campo Grande, MS, in 2016. **Results:** Of the 68 water samples collected after the reverse osmosis process, three (4.41%), and of the 58 samples collected in the reuse room, six (10.52%) presented endotoxin results above the amount allowed by the legislation (0.25 EU\mL). **Conclusions:** Results indicate membrane involvement of the reverse osmosis process, and, or presence of biofilm in the pipes, and inefficiency of the cleaning process of the reservoir, pipes, and machines disinfection process.

KEYWORDS: Chronic Renal Failure; Endotoxins; Dialysis Water; Public Health Surveillance

RESUMO

Introdução: A doença renal crônica caracteriza-se pela perda da função dos rins levando a um desequilíbrio funcional, fisiológico e biológico. Um dos tratamentos necessários ao paciente renal crônico é a hemodiálise onde a qualidade da água é de fundamental importância para reduzir riscos aos pacientes. Através da Resolução RDC nº 11, de 13 março de 2014, a Agência Nacional de Vigilância Sanitária (Anvisa) estabeleceu a pesquisa de endotoxinas, cuja contaminação por via venosa poderá causar vários agravos à saúde dos pacientes. **Objetivo:** Identificar endotoxinas em água purificada para hemodiálise. **Método:** Trata-se de um estudo transversal descritivo quantitativo para verificação da presença de endotoxinas em 126 amostras de água, coletadas em dois pontos (pós-osmose reversa e sala de reúso) nas seis clínicas de terapia renal substitutiva, pela Vigilância Sanitária Estadual, analisadas no LACEN, em Campo Grande, Mato Grosso do Sul, 2016. **Resultados:** Das 68 amostras de água coletadas após o processo de osmose reversa, três (4,41%) e das 58 amostras coletadas na sala de reúso, seis (10,34%) apresentaram resultados de endotoxinas acima do valor permitido pela legislação (0,25 EU\mL). **Conclusões:** Os resultados encontrados indicam comprometimento da membrana do processo de osmose reversa e, ou presença de biofilme nas tubulações, ineficácia do processo de limpeza do reservatório, tubulações, e desinfecção das máquinas.

PALAVRAS-CHAVE: Insuficiência Renal Crônica; Endotoxinas; Água para Diálise; Vigilância em Saúde Pública

INTRODUCTION

Chronic Kidney Disease (CKD) is recognized worldwide as a public health problem. It is a complex and multifactorial pathology, characterized by progressive and irreversible reduction of glomerular filtration, leading to chronic renal failure (CRF)[1,2].

The number of chronic kidney patients on dialysis increases steadily in Brazil, while in the United States and Japan there is a trend toward stabilization. In Brazil, there are approximately 750 renal replacement therapy units, with an estimated 122,825 chronic renal patients, 90.6% of which currently undergoing hemodialysis. The state of Mato Grosso do Sul presents the sixth highest prevalence in the country, with 678 patients per million inhabitants. In the state capital, Campo Grande, there are six dialysis facilities, with approximately 1,000 patients[3].

Hemodialysis is an extracorporeal process used to normalize the electrolyte balance and to remove toxic substances from the body with the use of an artificial kidney (or dialyzer) and dialysis solution, mainly composed of water[1]. Patients on hemodialysis undergo the procedure about three times a week, in a period of three to five hours, with the expenditure of 120 liters of water per session[4,5].

Water quality is of paramount importance to reduce risks to patients. The Brazilian National Agency of Health Surveillance (Anvisa) established a survey of endotoxins as one of the biological parameters that should be controlled in hemodialysis water through the Resolution of the Collegiate Board of Directors (RDC) n. 11, of March 13, 2014[6]. Endotoxins are pyrogenic substances of lipopolysaccharide nature, resulting from the autolysis of cell walls of Gram-negative bacteria, whose intravenous administration can cause several physiological changes in patients[3,7].

Even at low concentrations, endotoxins contribute to a chronic inflammatory response, since they are capable of stimulating the expression of cytokines and proinflammatory mediators in monocytes and macrophages[8]. They can also cause acute complications such as: pyrogenic reactions, headache, nausea, cramps and cardiovascular problems[9]. They are related to prolonged morbidity in patients on renal replacement therapy[10,11,12].

Research has shown that the use of ultrapure water in the dialysis process reduces the inflammatory reactions in hemodialysis patients due to the absence of endotoxins[13].

Although dialysis services control water quality, studies show deficiencies in some control stages, with detection of endotoxins and patient mortality[14,15].

Considering the risk of morbidity and mortality for chronic renal patients, this study aims to identify the presence of endotoxins in hemodialysis water.

METHOD

This is a descriptive cross-sectional quantitative study with the purpose of evaluating the presence of endotoxins in water for hemodialysis within the monitoring program carried out by the state Health Surveillance body of Mato Grosso do Sul (MS), from February to December 2016.

A total of 126 samples of purified water were collected at two points, 68 (53.97%) after the reverse osmosis treatment and 58 (46.03%) at the reuse room faucet, by the state Health Surveillance body, in six hemodialysis clinics located in the municipality of Campo Grande, MS, coded with the following letters of the alphabet: A, B, C, D, E, and F. The collection was performed monthly, except in the clinics with non-conforming results, where there was a new collection after correction procedures.

The inclusion criterion was the monitoring program for the year 2016 of clinics based in the municipality of Campo Grande. Clinics based in other municipalities of the state were excluded from the study.

The endotoxin analysis was performed in the Central State Public Health Laboratory (LACEN), using the *Lymulus amebocyte Lysate* methodology (LAL, brand Endosafe), with gel formation, indicating the presence of endotoxin in the sample containing a quantity equal to or greater than its sensitivity (y). The detection limit of the method was 0.06 EU/mL. Endotoxin levels were assessed according to the maximum allowable value (0.25 EU/mL) by Anvisa RDC n. 11/2014.

The data were tabulated in Excel and analyzed through descriptive statistics with absolute and relative frequency calculation.

RESULTS AND DISCUSSION

The risks to which chronic renal patients undergoing hemodialysis are submitted are directly related to water quality[16]. Aiming to prevent these risks, from the 1970s onwards, the use of potable water was replaced by water purified through the deionization and reverse osmosis processes[17].

Of the 68 water samples collected after the osmosis process, three (4.54%), and of the 58 samples collected in the reuse room, six (10.34%) presented results above the value allowed by the current legislation (0.25 EU/mL) (Figure 1). Non-conforming results were identified in two (33.33%) of the six clinics evaluated (Figure 2).

Of the total number of samples (68) of post-osmosis, 65 (95.60%) complied with current legislation and three (4.40%) presented endotoxins with a concentration of 0.5 EU/mL, not in compliance with the legislation (Table 1).

Clinic A presented non-conforming results at the point of collection after reverse osmosis, characterizing a defect in the osmosis

membrane in the months of March, October and November. We observed that only in October there was growth of heterotrophic bacteria below the limit allowed by the legislation.

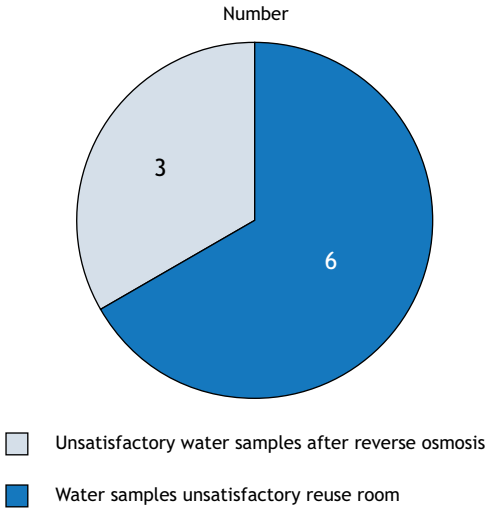

Figure 1. Distribution of unsatisfactory samples per point of collection. Campo Grande, MS, 2016.

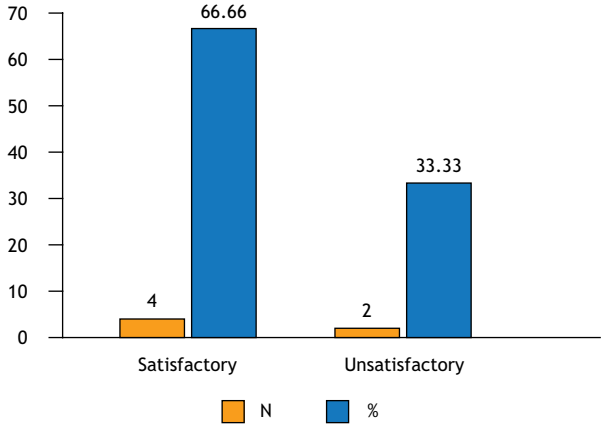

Figure 2. Hemodialysis clinics with satisfactory and unsatisfactory results for endotoxins. Campo Grande, MS, 2016.

In the samples collected in the reuse room, 52 (89.7%) complied with the legislation and six (10.30%) were unsatisfactory (Table 2). The six unsatisfactory results for endotoxins were all from Clinic D (37.5%), in the months of: February (one sample), May (one sample), June (two samples) and July (two samples). In these results, three samples had counts of heterotrophic bacteria above the value tolerated by the current legislation.

The tubing system of dialysis machines can promote microbial growth and biofilm formation, which acts as a source of endotoxins. The accumulation of endotoxins can occur after the water has circulated through the tubing system, which justifies the highest percentage (37.50%) of endotoxins in the water of the reuse room.

Biofilm is a community structure of microbial cells protected by a polysaccharide matrix synthesized by the bacteria themselves. Biofilm formation is an important factor for the onset of infectious diseases, since its release leads to increased endotoxin levels and resistance to disinfection procedures[18].

The presence of endotoxins with levels below what is established as tolerable by the legislation (38.00%) is also of concern, since studies have shown a higher mortality risk for chronic renal patients with any amount of endotoxin present in the water and persistence of inflammatory processes[18].

To avoid these risks during the hemodialysis process, Europe and Japan are currently using dialysate produced with ultrapure water, which has count limits for bacteria lower than 0.1 CFU/mL and endotoxin levels below 0.03 EU\mL[19].

Several studies on water collected after the reverse osmosis process indicated results for endotoxins above those found. Shahryari et al., in 2016, identified 17.5% of the samples with 0.5 to 2.0 EU/mL, 77.5% with values < 0.5 EU/mL and 5.0% with values > 2.0 EU\mL, in Iran[20]; Figel et al., in 2015, detected 15.0% of the samples with values above 2.0 EU\mL, in Curitiba, Brazil[21]; Soares et al., in a municipal hospital of Governador Valadares, Brazil, in 2016, found 10.0% of the endotoxin samples above the value allowed by the current legislation[22].

Table 1. Distribution of endotoxin samples in post-reverse osmosis water from dialysis clinics. Campo Grande, MS, 2016.

Dialysis Clinics	Nº of samples	Nº and % of samples with endotoxin concentration		
		Absence of endotoxins	0.25EU\m L	0.5 EU\mL
Clinic A	11	1 (9.10)	7 (63.60)	3 (27.30)
Clinic B	10	10 (100.00)	0.0	0.0
Clinic C	10	9 (90.00)	1 (10.0)	0.0
Clinic D	15	11 (73.30)	4 (26.70)	0.0
Clinic E	11	11 (100.00)	0.0	0.0
Clinic F	11	10 (90.90)	1 (9.10)	0.0
Total	68	52 (76.50)	13 (19.10)	3 (4.40)

Table 2. Number and percentage of endotoxin samples in water from the reuse room at hemodialysis clinics. Campo Grande, MS, 2016.

Dialysis Clinics	Nº of samples	Nº and % of samples with endotoxin concentration		
		Absence	0.25 EU\mL	0.5 EU\mL
Clinic A	*	*	*	*
Clinic B	10	8 (80.00)	2 (20.0)	0.0
Clinic C	10	8 (80.00)	2 (20.0)	0.0
Clinic D	16	5 (31.25)	5 (31.20)	6 (37.50)
Clinic E	11	10 (90.90)	1 (9.10)	0.0
Clinic F	11	10 (90.90)	1 (9.10)	0.0
Total	58	41 (70.70)	11 (19.00)	6 (10.30)

* Reuse room was shut down

Considering that some time elapses between the collection, the release of the result and the enforcement of actions, patients were exposed to water that was considered inadequate for some time. The minimization or elimination of this risk would only be possible with the adoption of additional measures, such as the use of biofilm-resistant tubing in the walls, the use of dialysis machines with filters for the production of ultrapure dialysis solution and the end of the reuse of dialyzers and lines, the most critical point in the whole process, because it invariably causes outbreaks of pyrogenicity in hemodialysis. Reuse is the only time the water comes into direct contact with the inner surface of the dialyzer membranes, allowing bacteria and endotoxins to be adsorbed where the blood will circulate in the next session of hemodialysis.

In the city of Campo Grande, MS, the control performed by the clinics and the program of dialysis water quality monitoring of the Health Surveillance body are performed according to the periodicity established by RDC n. 11/2014 of Anvisa[6]. However, when analyzing the data collected in 2016, there were clinics that did not comply with the legislation, leading to potential risks to the health of the patients treated there. These clinics were notified by the Health Surveillance body and oriented to adopt corrective measures. After the recommended adjustments, new samples were collected for analysis.

Clinics with water samples in compliance with the legislation demonstrated that the cleaning and disinfection procedures in the treatment system were efficient, with absence of biofilm.

This study has limitations because it has no technical information regarding the clinics, like number of patients, time of operation, information regarding infections, endotoxinemia and mortality.

CONCLUSIONS

The presence of endotoxin in hemodialysis water characterizes problems in the membrane used in the reverse osmosis process and/or the presence of biofilm in the piping. This indicates the need to reduce the risks by replacing the membrane filters, periodic cleaning of the reservoir, piping and disinfection of machines.

A monitoring program is an instrument to ensure that the dialysis services perform the procedures in accordance with current legislation and identify needs for additions or amendments in the legislation.

The results suggest the need to change the legislation and regulation of the water treatment process, replacing reverse osmosis with the ultrapurification system to reduce risks and maintain the health stability of chronic renal patients.

REFERÊNCIAS

1. Riella MC. Princípios de nefrologia e distúrbios hidroeletrolíticos. 5a ed. Rio de Janeiro, RJ: Guanabara Koogan; 2010.
2. Souza BGA. Qualidade microbiológica de água tratada para hemodiálise e caracterização de bactérias patogênicas [tese]. Goiânia: Faculdade de Nutrição da Universidade Federal de Goiás; 2015.
3. Sesso RC, Lopes AA, Thomé FS, Lugon JR, Martins CT. Inquérito brasileiro de diálise crônica 2016. J Bras Nefrol. 2017;39(3):261-66. https://doi.org/10.5935/0101-2800.20160009
4. Secretaria de Estado de Saúde (BR). Atenção transdisciplinar ao renal crônico: manual para abordagem de pacientes em tratamento hemodialítico. Campo Grande, MS: Secretaria de Estado de Saúde; 2011.
5. Liberato GS. Aplicação do plasma elétrico no controle de qualidade bacteriológica da água de osmose reversa utilizada em serviços de hemodiálise [dissertação]. São José dos Campos: Instituto de Pesquisa e Desenvolvimento da Universidade do Vale da Paraíba; 2017.
6. Agência Nacional de Vigilância Sanitária - Anvisa. Resolução da Diretoria Colegiada - RDC Nº 11, de 13 de março de 2014. Dispõe sobre os Requisitos de Práticas de Funcionamento para os Serviços de Diálise e dá outras providências, Diário Oficial da União. 14 mar 2014.

7. Tristão AMV. Qualidade da água nas clínicas de hemodiálise no estado de Mato Grosso do Sul [dissertação]. Campo Grande: Universidade Federal de Mato Grosso do Sul; 2014.
8. Mcintyre CW, Harrison LEA, Eldehni MT. Circulating endotoxemia: A novel fator in systemic inflammation and cardiovascular disease in chronic kidney disease. Clin J Am Soc Nephrol. 2011;6(1):133-41. https://doi.org/10.2215/CJN.04610510
9. Buzzo ML, Bugno A, Almodovara AB, Kira CS, Carvalho MFH, Souza A, Scorsafavam A. A importância de programas de monitoramento da qualidade da água para diálise na segurança dos pacientes. Rev Inst Adolfo Lutz. 2010;69(1):1-6.
10. Câmara Neto HF. A tragédia da hemodiálise 12 anos depois: poderia ela ser evitada? [tese]. Recife: Centro de Pesquisas Aggeu Magalhães da Fundação Oswaldo Cruz; 2011.
11. Suzuki MN. Qualidade da água tratada para hemodiálise e intercorrências clínicas apresentadas pelos pacientes em tratamento: enfoque para metais e agentes microbiológicos [tese]. Ribeirão Preto: Escola de Enfermagem da Universidade de São Paulo; 2016.
12. Ibrahim M, Behairy M, El-Ashry, Mostafa AE. Cardiovascular risk of circulating endotoxin level in prevalente hemodialysis patients. Egyptian Heart J. 2017;70(1):27-33. https://doi.org/10.1016/j.ehj.2017.06.003.
13. Di Iori B, Di Micco L, Bruzzese D, Nardone L, Russo L, Formisano P, D'Esposito V. Ultrapure dialysis water obtained with additional ultrafiltre may reduce inflammation in patients on hemodialysis. J Nephrol. 2017;30(6):795-801. https://doi.org/10.1007/s40620-017-0422-x
14. Jesus GP, Almeida AA. Principais problemas gerados durante a terapia de hemodiálise associados à qualidade da água. Rev Eletr Atual Saúde. 2016;3(3):41-52.
15. Ferreira JAB, Nóbrega HN, Freitas HR, Moura DC, Marin VA, Sejas CGF. Águas de hemodiálise: controle de qualidade em saúde. Rev Bras Med. 2015;72(11):480-85.
16. Harrison LEA, Burton JO, Szeto C, Li PKT, Mclntyre CW. Endotoxemia in haemodialysis: a novel fator in erythropoetin resistance? Plos One. 2012;7(6):38-40. https://doi.org/10.1371/journal.pone.0040209
17. Ramirez SS. Avaliação do impacto da qualidade da água em parâmetros laboratoriais indicativos de bem estar clínico em pacientes submetidos à hemodiálise no Estado do Rio de Janeiro [dissertação]. Rio de Janeiro: Instituto Nacional de Controle de Qualidade em Saúde da Fundação Oswaldo Cruz; 2011.
18. Feroze U, Kalantar-Zadeh K, Sterling KA. Examining associations of circulating endotoxin with nutritional status, inflammation and mortality in hemodialysis patients. J Ren Nutr. 2012;22(3):317-26. https://doi.org/10.1053/j.jrn.2011.05.004
19. Ferreira JAB, Nobrega HN, Vieira VV, Abrantes SMP. Diversidade Genética e produção de biofilme de amostras de *P. aeruginosa* isoladas da água utilizada em Unidades de Terapia Renal Substitutiva. Rev Analítica. 2013;(65):56-70.
20. Shahryari A, Nikaeen M, Hatamzadeh M, Vahid Dastjerdi M, Hassanzadeh A. Evaluation of bacteriological and chemical quality of dialysis water and fluid in Isfahan, Central Iran. Iran J Public Health. 2016;45(5):650-56.
21. Figel IC, Dalzoto PR, Pimentel ICH. Qualidade microbiológica da água e dialisato em clínicas de hemodiálise do Sul do Brasil. Rev Inst Adolfo Lutz. 2015;74(1):66-70.
22. Soares NAMS, Barony FJA, Cruz FM, Pena LFR. Sistema de tratamento de água: um estudo de caso em um serviço de diálise no município de Governador Valadares, MG. In: VII Congresso Brasileiro de Gestão Ambiental; Campina Grande, Brasil. Campina Grande, PB: Universidade Federal de Campina Grande/Instituto Brasileiro de Estudos Ambientai e de Saneamento; 2016.

Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.