The level of endotoxins in hemodialysis water and dialysate in Lithuanian hemodialysis centers

Inga Skarupskienė, Inga Arūnė Bumblytė, Donatas Tamošaitis, Jūratė Venterienė, Vytautas Kuzminskis
Department of Nephrology, Kaunas University of Medicine, Lithuania

Key words: endotoxin; hemodialysis; water; dialysate; Limulus amebocyte lysate test.

Summary: The composition and quality of the dialysis fluid play an important role in the modulation of dialysis-related complications. During hemodialysis, patient’s blood has a contact with dialysate through a semipermeable membrane. Bacterial endotoxins can pass through the membrane pores into the patient’s blood and cause a silent chronic microinflammation. The aim of this study was to determine the level of endotoxins in hemodialysis water and dialysate in Lithuanian hemodialysis centers. Dialysis water (n=50) and dialysate (n=50) were collected from 91% (n=50) of all hemodialysis centers. The presence of bacterial endotoxins was evaluated using a sensitive Limulus amebocyte lysate test, which detects intact lipopolysaccharides. The level of endotoxins was lower than 0.25 EU/mL in 43 (86%) dialysis water samples and in 46 (92%) dialysate samples, and complied with the recommendations of the European Pharmacopoeia and the European Best Practice Guidelines for pure dialysis fluid. The dialysate of 39 (78%) Lithuanian hemodialysis centers complied with the definition of an ultrapure dialysis fluid. The water and dialysate were of insufficient quality in 14% and in 8% of Lithuanian hemodialysis centers, respectively, and this could be improved by the establishment of routine investigation of endotoxins.

Introduction

Patients with renal failure are exposed to a higher volume of water in their lifetime as compared to general population (1–2). A patient on hemodialysis (HD) is exposed to approximately 360 liters of water per week whereas a healthy person is typically exposed only to 14 liters per week. During HD procedure, patient’s blood has a contact with dialysate through a semipermeable membrane. The composition of the dialysis fluid plays an important role in the modulation of dialysis-related complications (1–4). Water, which is used for dialysis, is not sterile. Microorganisms cannot pass through the membrane pores, but bacterial endotoxins (ET) (lipopolysaccharides), exotoxins, bacteria-derived short DNA fragments can migrate across the dialysis membrane into the patient’s blood. These microbial contaminants trigger and maintain a silent chronic microinflammation in HD patients by activating monocytes and macrophages and inducing the release of proinflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor) (3, 5–8).

Current literature also indicates a positive effect of ultrapure dialysate on the morbidity and mortality of HD patients. A recent study revealed that serum β2-microglobulin level is a significant predictor of mortality in maintenance HD patients (17). Ultrapurity of dialysis fluid is recognized worldwide as a necessary step for improving overall dialysis quality (18).

Today there are an increasing number of clinical studies showing the impact of dialysis fluid quality on important parameters of HD patients. The use of ultrapure dialysate is associated with a lower concentration of inflammatory markers (1, 9–12), improvement of nutritional status (10), an increase in responsiveness to erythropoietin (1, 10, 13, 14) and hemoglobin level (9). Furthermore, it improves iron utilization (13), slows down the decline of residual renal function (1, 10), decreases dyslipidemia and oxidative stress (11, 15), plasma level of β2-microglobulin (9, 10, 12), and incidence of β2-microglobulin amyloidosis (12, 16). A recent study revealed that serum β2-microglobulin level is a significant predictor of mortality in maintenance HD patients (17). Ultrapurity of dialysis fluid is recognized worldwide as a necessary step for improving overall dialysis quality (18).

The avoidance of complications arising from water and dialysate contaminants requires a constant attention to water and dialysate quality. The European Best...
Practice Guidelines for HD give detailed recommendations how to test HD water and dialysis fluid. For standard HD in Europe, the maximum contamination level for water and dialysis fluid has been defined by the European Pharmacopoeia as <100 colony forming units (CFU/mL) and <0.25 ET units (EU/mL). Dialysis fluid for hemodiafiltration (HDF) and high-flux dialysis modalities should be ultrapure (<0.1 CFU/mL for bacteria, <0.03 EU/mL for ET). Water microbiology and dialysate should be monitored at least monthly (19). In Lithuania, bacteriometry of HD water and dialysate is performed monthly according to national recommendations, but the level of ET is not routinely tested.

The aim of this national study was to determine the level of ET in HD water and dialysate in Lithuanian HD centers.

**Material and methods**

The study was performed in majority (n=50; 91%) of Lithuanian HD centers in the year 2009.

The samples for ET analysis (10 mL of water after reverse osmosis and 10 mL of dialysate from each HD center) were collected aseptically in pyrogen-free syringes and stored at −20°C. The analysis of samples was performed in the laboratory of Ghent University Hospital (Ghent, Belgium). The presence of bacterial ET was evaluated using a sensitive Limulus amebocyte lysate (LAL) test, which detects intact lipopolysaccharides (membrane components of gram-negative bacteria).

**Results**

In the year 2009, there were 55 HD centers with 1400 ambulatory HD patients in Lithuania. The level of ET was evaluated in water (n=50) (Fig. 1) and dialysate (n=50) (Fig. 2) of 50 (91%) HD centers. Five HD centers were not included into this study.

Overall, the level of ET varied from <0.003 to 7.855 EU/mL in HD water and from <0.003 to 7.441 EU/mL in dialysate. The level of ET was lower than 0.25 EU/mL in 43 (86%) samples of HD water and in 46 (92%) samples of dialysate. Therefore, it complied with the recommendations of the European Pharmacopoeia and the European Best Practice Guidelines (EBPG) for pure dialysis fluid. The dialysate in 39 (78%) HD centers and HD water in 28 (56%) HD centers in Lithuania complied with the definition of

---

**Fig. 1.** Level of endotoxins in water of hemodialysis centers (n=50) in Lithuania

**Fig. 2.** Level of endotoxins in dialysate of hemodialysis centers (n=50) in Lithuania
ultrapure dialysis fluid.

The water and dialysate were of insufficient quality (the level of ET was higher than 0.25 EU/mL in 14% and in 8% of HD centers, respectively, in Lithuania.

Discussion

Our present study is a national survey of the dialysis fluid quality. In 2009, Lithuania had 55 HD centers and approximately 1400 HD patients (the data of Lithuanian Association of Nephrology, Dialysis, and Transplantation). We investigated the water and dialysate samples in majority (n=50, 91%) of HD centers in Lithuania, where 93% (n=1304) of all HD patients were treated. The results showed that pure dialysis water was found in 86% of HD centers and pure dialysate in 92% of HD centers. Ultrapure HD water and dialysate was found in 56% and in 78% of HD centers, respectively. Our first attempt to investigate the quality of dialysis fluid in Lithuania was made in 2007. At that time, it was a small study investigating ET level in dialysis water and dialysate. We investigated dialysis water (n=6) and dialysate (n=14) in 6 HD centers in 3 different cities of Lithuania. The level of ET was lower than 0.25 EU/mL in all samples of HD water (n=6) and dialysate (n=14) and complied with the recommendations of the EBPG for pure dialysis fluid. One sample of water (16.7%) and two samples of dialysate (14.3%) had the level of ET more than 0.03 EU/mL.

The data on the level of ET in dialysis fluid vary in different countries. El-Koraie et al. investigated the bacteriological quality of dialysis fluid in 2 HD units in Alexandria, Egypt, in 2005 (21). A total of 321 samples were collected from treated water and dialysate. The LAL assay showed that the level of ET exceeded 0.25 EU/mL in all the samples. The SIN survey on quality control of dialysis water in Italy revealed that only 60% of 297 HD units investigated were performing the test for ET in dialysis water (22). Lamas et al. found poor compliance with ET criteria over a period of one year at two HD units in Spain (23). A range of ET was 1.83–2.645 and 0.05–60.87 EU/mL in dialysate from units A and B, respectively. In our study, the results were better: the level of ET ranged from <0.003 to 7.855 and from <0.003 to 7.441 EU/mL in HD water and dialysate, respectively.

The standard for dialysis water and dialysis fluid for the ET level recommended by the Japanese Society for Dialysis Therapy (JSDT) is strictest (<0.05 EU/mL) in the world. The JSDT surveyed all dialysis facilities for bacteriological quality of dialysis fluid in 2006 and 2007. The JSDT collected the data on ET levels, bacterial count, and usage of endotoxin-retentive filters. The standard for ET level in dialysis fluid was achieved in 89% of samples in 2006 and correspondingly in 93.6% of samples in 2007 (24). Dialysis fluid with ET below the detection level (usually <0.001 EU/mL) is used for online HDF in Japan (25). The survival rate of dialysis patients in Japan has been proven to be extremely high among the worldwide outcome study. The high quality of dialysis fluid, which was proven in the JSDT surveys, might play an important role in the excellent survival rate in Japan (24).

A few recent reports confirmed the close relationship between the water quality and patient survival. The 1-year survival rate of the patients treated with dialysis fluid with an ET level of 0.1–0.25 EU/mL was significantly lower than that of the patients who were treated with dialysis fluid with an ET level of <0.001 EU/mL (26). In the Dialysis Outcomes and Practice Patterns Study (DOOPS), it was also reported that an ET level of >0.12 EU/mL was associated with a significantly higher risk for all-cause and cardiovascular death (27). In Lithuania, there are no studies designed to test an impact of HD water and dialysate quality on HD patient survival. It could be one of the aims for further investigations on quality of HD fluids.

At present time in Lithuania, HD centers are able to investigate routinely only a quantity of bacteria in HD water and dialysate. Our study showed that ET level in HD water and dialysate was too high in 14% and in 8% of Lithuanian hemodialysis centers, respectively. It is necessary to establish additional monitoring of ET level in dialysis fluids in the near future. The International Organization for Standardization (ISO) is currently working on the development of the guidance document (guidance for the preparation and quality management of fluids for HD and related therapies), which is expected to be available in 2010 (28). It will be the basis for corrections of now existing recommendations for water and dialysate quality in Lithuania.

Conclusions

1. Samples of dialysis water from 86% of hemodialysis centers and dialysate samples from 92% of hemodialysis centers in Lithuania complied with the recommendations of the European Pharmacopoeia and the European Best Practice Guidelines for pure dialysis fluid.

2. The dialysate from 78% of hemodialysis centers and water from 56% of hemodialysis centers in Lithuania complied with the definition of an ultrapure dialysis fluid.

3. The level of endotoxins in dialysis water and dialysate should be routinely monitored in Lithuania.
Endotoksinų kiekis hemodializei naudojamame vandenyje ir dializės tirpale
Lietuvos hemodializijų centruose

Inga Skarupskienė, Inga Arūnė Bumblytė, Donatas Tamošaitis,
Jūratė Venterienė, Vytautas Kuzmins
Kauno medicinos universiteto Nefrologijos klinika

Raktažodžiai: endotoksinai, hemodializė, vanduo, dializės tirpalas, LAL testas.

Santrauka. Dializės tirpalų sudėtis ir kokybė yra labai svarbi, nes gali turėti įtakos su dialize susijusioms komplikacijoms. Hemodializės procedūros metu paciento kraujas per pusiau pralaidžiąją membraną kontaktuoja su dializės tirpalu. Bakterijų endotoksinai gali patekti per membranos poras į ligonio kraują, skatinti bei palaikyti lėtų uždegimų reakciją.

Tyrimo tikslas. Ištirti endotoksinų kiekį hemodializei naudojamame vandenyje ir dializės tirpale visuose Lietuvos hemodializijų centruose.

Tyrimo metuogi ir metodai. Endotoksinų kiekį ištirtas 50 (91 proc.) Lietuvos hemodializės centrų dializei naudojamame vandenyje (n=50) ir dializės tirpale (n=50). Endotoksinų kiekį nustatytas LAL (Limulus amebocyte lysate assay) testu.

Rezultatai ir išvados. Mažesnis nei 0,25 EV/ml endotoksinų kiekis nustatytas 43 (86 proc.) hemodializės vandens ir 46 (92 proc.) dializės tirpalo mėгинiuose ir atitiko minimalius Europos farmakopėjos ir Europos geros praktikos gairių reikalavimus švariamis tirpalams. 39 (78 proc.) hemodializės centrų dializės tirpalo ir 28 (56 proc.) hemodializės centrų endotoksinų kiekis atitiko ypatingai švaraus tirpalo Europos geros praktikos rekomendacijas. Nepakankamos kokybės vanduo ir dializės tirpalas rastas atitinkamai – 14 ir 8 proc. Lietuvos hemodializės centruose. Hemodializės tirpalų kokybę galima pagerinti nuolat tiriandiant endotoksinų kiekį.

References
1. Hoenich NA, Ronco C, Levin R. The importance of water quality and haemodialysis fluid composition. Blood Purif 2006;24:11-8.
2. Bommer J, Jabert BL. Ultrapure dialysate: facts and myths. Semin Dial 2006;19(2):115-9.
3. Hoenich NA, Levin R, Ronco C. How do changes in water quality and dialysate composition affect clinical outcomes? Blood Purif 2009;27:11-5.
4. Masakane I. Review: clinical usefulness of ultrapure dialysate – recent evidence and perspectives. Ther Apher Dial 2006;10(4):348-54.
5. Schindler R, Beck W, Deppisch R, Aussieker M, Wilde A, Goh I, et al. Short bacterial DNA fragments: detection in dialysate and induction of cytokines. J Am Soc Nephrol 2004;15:3207-14.
6. Handelman GJ, Megdal PA, Handelman SK. Bacterial DNA in water and dialysate: detection and significance for patient outcomes. Blood Purif 2009;27:81-5.
7. Bossola M, Sanquineti M, Scriboni D. Circulating bacterial-derived DNA fragments and markers of inflammation in chronic hemodialysis patients. Clin J Am Soc Nephrol 2009;4:379-85.
8. Schindler R. Clinical effect of purification of dialysis fluids, evidence and experience. Blood Purif 2009;27(Suppl 1):20-2.
9. Azirano K, Nomura K, Motoyama T, Matsumo Y, Matsuoka K, Miyazu R, et al. Ultrasound of ultrapure dialysate in reduction of chronic inflammation during hemodialysis. Blood Purif 2004;22(2):26-9.
10. Ledebo I. Ultrapure dialysis fluid – how pure is it and do we need it? Nephrol Dial Transplant 2007;22:20-3.
11. Schiffl H, Lang SM. Effects of dialysis purity on uremic dyslipidemia. Ther Apher Dial 2006;14(1):5-11.
12. Furuya R, Kumagai H, Takahashi M, Sano K, Hishida A. Ultrapure dialysate reduces plasma levels of beta2-microglobulin and pentosidine in hemodialysis patients. Blood Purif 2005;23(4):311-6.
13. Hsu PY, Lin CL, Yu CC, Chien CC, Hsiau TG, Sun TH, et al. Ultrapure dialysate improves iron utilization and erythropoietin response in chronic hemodialysis patients – a prospective cross-over study. J Nephrol 2004;17(5):693-700.
14. Go I, Takemoto Y, Tsuchida K, Sugimura K, Nakatani T. The effect of ultrapure dialysate on improving renal anemia. Osaka City Med J 2007;53:17-23.
15. Oka Y, Miyazaki M, Takatsu S, Kunitomo K, Kumakura Y, Matsuda H, et al. Lowering of oxidative stress in hemodialysis patients by dialysate cleaning: in relation to atherosclerosis. Ther Apher Dial 2004;8:313-9.
16. Lonnenman G, Koch KM. Beta(2)-microglobulin amyloidosis: effects of ultrapure dialysate and type of dialyzer membrane. J Am Soc Nephrol 2002;13(Suppl 1):S72-7.
17. Okuno S, Ishimura E, Kohno K, Fujino-Kato Y, Maeno Y, Yamakawa T, et al. Serum beta2-microglobulin level is a significant predictor of mortality in maintenance haemodialysis patients. Nephrol Dial Transplant 2009;24:571-7.
18. Canaud BM. Changing paradigms of renal replacement therapy in chronic kidney disease patients: ultrapure dialysis fluid and high-efficiency hemodiafiltration for all? Kidney Int 2009;76:591-3.
19. The EBPG Expert Group on Haemodialysis. European best...
practice guidelines for haemodialysis. Dialysis fluid purity. Nephrol Dial Transplant 2002;17(Suppl 7):45-62.
20. Skarupskienė I, Bumblytė IA, Kuzminskis V. The level of endotoxins in hemodialysis water and dialysate. Medicina (Kaunas) 2007;43(Suppl 1):81-4.
21. El-Koraie AF, Hazzah WA, Abbass AA, El-Shazly SA. Bacteriological monitoring of dialysis fluid in 2 hemodialysis units in Alexandria, Egypt. Saudi Med J 2007;28(8):1234-8.
22. Bonfant G, Belfanti P, Cappelli G, Alloatti S. SIN survey on dialysis water controls in Italy. G Ital Nefrol 2005;22:241-5.
23. Lamas JM, Alonso M, Sastre F, Saavedra JA, Garcia-Trio G, Palomares L. Dialysate bacteriological quality in a health district. Nefrologia 2007;27(2):191-5.
24. Masakane I, Takemoto Y, Nakai S, Tsubakihara Y, Akiba T, Watanabe Y, et al. Bacteriological water quality in the central dialysis fluid delivery system from the survey of the Japanese Society for Dialysis Therapy. Blood Purif 2009;27(Suppl 1):11-6.
25. Yamashita AC, Sato T. Central online hemodiafiltration in Japan: management of water quality and practice. Blood Purif 2009;27(Suppl 1):50-5.
26. Masakane I, Nakai S, Akiba T, Watanabe, Iseki K, Tsubakihara Y. Bacteriological water quality has a strong impact on the outcome of chronic dialyzed patients. J Am Soc Nephrol 2008;19:693A.
27. Hasegawa T, Pisoni RL, Bragg-Gresham JL, Fukuhara S, Akizawa T, Saito A, et al. Dialysate endotoxin level and mortality of hemodialysis patients in the United States based on the DOPPS. J Am Soc Nephrol 2008;19:70A.
28. Ward RA. Worldwide guidelines for the preparation and quality management of dialysis fluid and their implementation. Blood Purif 2009;27(Suppl 1):2-4.

Received 15 June 2010, accepted 6 August 2010

Medicina (Kaunas) 2010; 46(8)