

EXPERIMENTAL INVESTIGATIONS

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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 ameobocyte 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 ameobocyte 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).

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 β 2-microglobulin (9, 10, 12), and incidence of β 2-microglobulin amyloidosis (12, 16). A recent study revealed that serum β 2-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

Correspondence to I. Skarupskienė, Department of Nephrology, Kaunas University of Medicine, Eivenių 2, 50028 Kaunas, Lithuania. E-mail: i.skarupskiene@mikrovisata.net

Adresas susirašinėti: I. Skarupskienė, KMU Nefrologijos klinika, Eivenių 2, 50028 Kaunas
El. paštas: i.skarupskiene@mikrovisata.net

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 amoebocyte 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

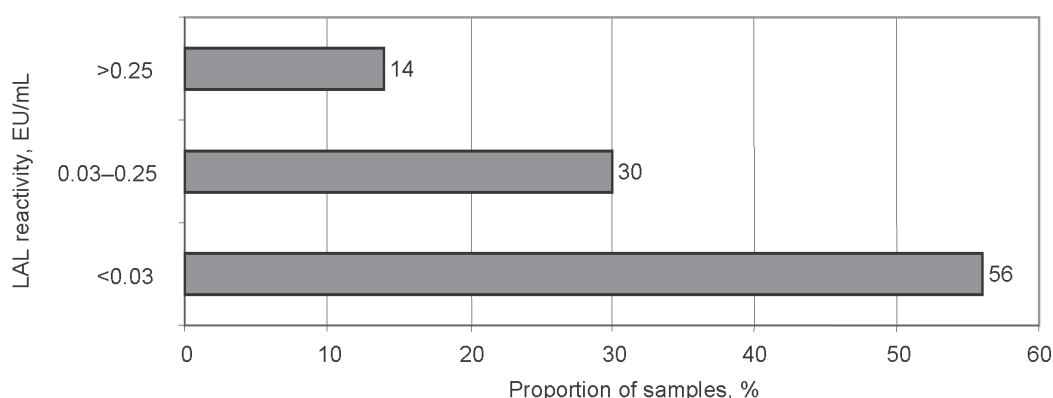


Fig. 1. Level of endotoxins in water of hemodialysis centers ($n=50$) in Lithuania

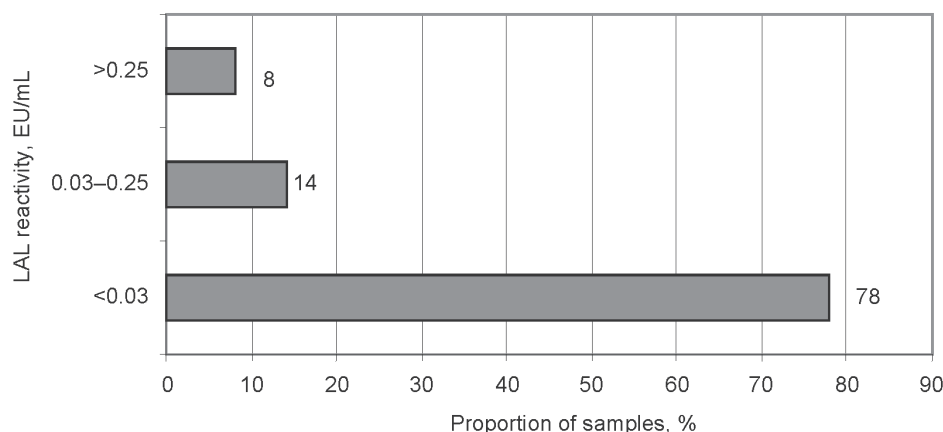


Fig 2. Level of endotoxins in dialysate of hemodialysis centers ($n=50$) in Lithuania

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 (20).

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 hemodializių centruose

Inga Skarupskienė, Inga Arūnė Bumblytė, Donatas Tamošaitis,
Jūratė Venterienė, Vytautas Kuzminskis

Kauno medicinos universiteto Nefrologijos klinika

Raktažodžiai: endotoksinais, 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 pralaidžią membraną kontaktuoja su dializės tirpalu. Bakterijų endotoksinais gali patekti per membranos poras į ligonio kraują, skatinti bei palaikyti lėtinę uždegiminę reakciją.

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

Tyrimo medžiaga ir metodai. Endotoksinų kiekis ištirtas 50 (91 proc.) Lietuvos hemodializės centrų dializei naudojamame vandenyje (n=50) ir dializės tirpale (n=50). Endotoksinų kiekis nustatytas LAL (*Limulus amoebocyte 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 mėginiuose ir atitiko minimalius Europos farmakopėjos ir Europos geros praktikos gairių reikalavimus švarems tirpalams. 39 (78 proc.) hemodializės centrų dializės tirpalo ir 28 (56 proc.) hemodializės centrų vandens endotoksinų kiekis atitiko ypač 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 tiriant endotoksinų kiekį.

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