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Water purity and inflammation in hemodialyzed patients

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ABSTRACT

During hemodialysis, blood comes in contact with a large volume of hemodialysis fluid. The dialysis fluid consists mainly of treated water. Since the purity of water has been linked to acute and long-term complications in hemodialysis patients, the aim of this study is to assess serum levels of some pro-inflammatory cytokines (IL-1β and TNF-α), acute phase proteins (C-reactive protein, ferritin and fibrinogen), and microbial analysis, with chronic inflammatory response in hemodialyzed patients that may be due to insufficient treatment of the water used for hemodialysis. Eighty subjects were included in the present study, 20 normal subjects as control and 60 hemodialysis patients from two different dialysis units (30 for each group) with age ranges from 28-75 years. All subjects were free from any inflammatory symptoms, according to the data received from the questionnaires (i.e. rise in body temperature, fever, chills, headache, loss of appetite and fatigue). IL-1β, TNF-α, CRP, ferritin and fibrinogen were significantly increased in all patients compared to normal controls. Based on microbiological analysis, water may be judged to be clean, even if in reality it is outside of standard recommendations. Although several causes contribute to the chronic micro-inflammatory state of uremic patients on dialysis, a small contamination of dialysate water with bacterial endotoxin may be an important factor to be considered.

Keywords: Acute phase proteins; Cytokines; Hemodialysis; *Pseudomonas aeurginosa*; Water purity.

INTRODUCTION

In hemodialysis, great amounts of water are used for diluting the concentrates to produce dialysis fluid. The water is produced on site by reverse osmosis units. Suitable chemical and microbiological water quality is essential for dialysis patients. Reverse osmosis units produce water of acceptable chemical quality that can be kept throughout the water system. The microbiological water quality, on the other hand, does not depend on the reverse osmosis unit but on the maintenance of the whole water system (Nystrand, 2008). Today, ultrapure water is included in most guidelines and recommended standards, but there

remains a need for harmonization between standards (Cappelli *et al.,* 2007; Ouseph *et al.,* 2007).

Although several International Organization for Standardization (ISO) measures are currently used in water preparation in order to achieve better harmonization (i.e. ISO 13958 [concentrates] (ISO 13958, 2009), ISO 13959 [water] (ISO 13959, 2009), ISO 26722 [water equipment] (ISO 26722, 2009) and ISO 11663 [ISO 11663, 2009] for dialysis fluid and substitution fluid), the number of different official recommendations for microbiological water quality is increasing. National authorities as well as international organizations publish recommendations; the most widespread recommendation is currently ISO standards 11663:2009. This is often used and referenced in areas where no local recommendations exist.

Water for dialysis represents a risk factor to the chronic inflammatory state documented in patients on end-stage renal disease (ESRD). The possibility of sustaining pro-inflammatory cytokines through microbial derived products, coming from dialysate or infused solutions, is enhanced by biofilm presence on piping and water treatment system or monitor components (Cappelli *et al.*, 2005; Skarupskiene *et al.*, 2007). A typical hemodialysis system includes a water treatment system; water treatment must eliminate chemical toxins and harmful microbial contamination. Most dialysis centers use reverse osmosis (RO) to effectively reduce bacteria and endotoxin levels (Pearson *et al.,* 1985).

Inflammation and infection seem to be important causes of morbidity and mortality in chronic kidney disease (CKD) patients (De Cal *et al.,* 2008). There is no uniform approach to assess the degree of severity of inflammation in individuals with kidney disease. Acute-phase reactants such as serum C-reactive protein (CRP) or ferritin serve as markers when serum levels are elevated during an acute episode of inflammation (Kalantar-Zadeh & Kopple, 2003; Pecoits-Filho *et al.,* 2003). A patient with a localized bacterial infection represents an excellent example of development of the acute phase response (Goldman & Claude, 2000).

The acute phase reactant response is initiated in response to trauma, inflammation, neoplasia, etc., and involves a release of cytokines (IL-1, IL-6, and TNF) from macrophages. These cytokines act on regulatory elements in hepatocyte genes, resulting in up-regulation of transcription of acute phase reactant proteins (fibrinogen, serum amyloid A protein, ceruloplasmin, and haptoglobin) and down-regulation of the transcription of other proteins, including albumin and transferring (so-called "negative acute phase reactants") (Casl, 1995).

SUBJECTS AND METHODS

Subjects

Patient samples

The study included 60 chronic renal failure patients on regular hemodialysis maintenance therapy using the Fresenius 4008B, USA, dialysis machine for the two units, and 20 healthy controls. The sixty patients were collected as 30 patients from two different units. The standard four-hour hemodialysis sessions were performed three times a week with cuprophane membranes, and citrate as the buffer solution. Water used for hemodialysis was subjected to purification and water treatment using reverse osmosis (RO). All subjects had no evidence of either inflammatory disease or malignancy and had no history of blood transfusion 3 months prior to sampling. Thirty minutes after beginning the dialysis session, ten milliliters of venous blood were collected directly from the outlet of the dialysis machine (once after circulating in the patient's blood and then out to the tube of the machine outlet tube) and divided into 2 parts A1 and A2.

A1 sample: containing 5 ml of blood, that was collected in dry clean centrifuge tubes and allowed to clot for 30 minutes at 37°C then centrifuged at 3000 rpm for 10 minutes and the serum was decanted, aliquoted and stored at -20°C to be thawed only once on demand.

A2 sample: containing 5 ml of blood that was collected in tubes containing sodium citrate as anticoagulant to obtain plasma which used for measuring of fibrinogen.

Blood samples were also collected from selected patients suspected to have bactraemia; the samples were inoculated in to the blood culture bottles (EMB blood culture medium). The bottle cultures were incubated at 35 - 37°C. Cultures were examined daily for 14 days, looking for above turbidity above the red cell layer, hemolysis, gas bubbles and growth on the surface layer.

Water samples

Municipal water was subjected to additional purification treatment in order to ensure appropriate quality. Water treatment systems that supply hemodialysis machines of both units included the same treatment components, such as particular sediment filters used to remove sediment from water, water softeners, carbon tanks, micro filters, ultraviolet disinfection units, reverse osmosis units, ultrafilters and storage tanks. Bacterial growth can occur on the filter and lead to subsequent bacteremia and/or pyrogenic reactions. Disinfection of the entire

RO unit, the delivery loop, and the hemodialysis machines, as well as water monitoring samples, was done monthly. Samples were taken from the treatment system tank's faucets. From each of the two dialysis units before sampling, a solution of sodium hypochlorite (100 mg NaOCl/l) was applied to faucets, and water was run for additional 2-3 minutes after treatment. Gloves were worn when collecting the samples to prevent skin bacteria from contaminating the samples. Water samples were collected in sample bottles (autoclavable polypropylene 1000 ml bottles) and analyzed for the sources of microbial contamination. Physico-chemical characteristics were also evaluated.

Bacteria isolated from patients and water were cultured and identified by morphological and biochemical examination using API 20E strips.

Methods

Blood samples

The concentrations of IL-1β and TNF-α were measured using Biosource international solid phase Enzyme Linked-Immuno-Sorbant Assay (ELISA). Briefly, for IL-1β, an antibody specific for humans, IL-1β has been coated onto the well of microtiter strips. Samples, including standards of known hIL-1β content, control specimens, and unknowns, are pipetted into these wells, followed by the addition of a biotinylated second antibody. During the first incubation, the hIL-1β antigen binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site. After removal of the excess second antibody, streptavidin-peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four- membrane sandwich. After a second incubation and washing to remove the entire unbound enzyme, a substrate solution is added which is acted upon by the bound enzyme to produce colour. The intensity of this colored product is directly proportional to concentration of hIL-1β present in the original specimen. A standard curve is plotted and IL-1β concentration in a sample is determined by interpolation from the standard curve. For TNF-α, an anti-TNF-α monoclonal coating antibody is adsorbed onto microwells. TNF-α present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated polyclonal anti-TNF-α antibody binds to TNF-á captured by the first antibody. Streptavidin-HRP binds to the biotin-conjugated anti-TNF-α. Following incubation, unbound biotin conjugated anti-TNF-α and Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of TNF-α present in the sample. The reaction is terminated by the addition of acid and absorbance is measured at 450 nm. A

standard curve is prepared from seven TNF-α standard dilutions and TNF-α sample concentration determined. Ferritin assay is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative determination of ferritin in human serum using an IMx system. Fibrinogen was measured as citrated plasma is brought to conjugation with a large excess of thrombin. Coagulation time depends on the fibrinogen content of the plasma.

Water samples

Water samples were examined for total heterotrophic bacteria using Reasoner's 2 Agar (R2A) (g/L) (Difco), total coliforms using m-Endo agar LES medium (Difco, USA) and fecal coliforms using m-FC agar medium (Difco, USA). Also, identification of founded bacteria was carried out by biochemical reactions using API 20E strip and *Pseudomonas aeurginosa* was identified using m-PAC agar (g/L) (BBL, USA). Physical parameters (Hydrogen ion concentration (pH value) was measured using pH meter (ORION) model 420A, electrical conductivity was measured using EC meter (WTW) Inolab, level 1 and total dissolved solids were determined by evaporating filtered water samples in a weighed dish on a steam bath and then dried in an oven at 103 ^{o}C to a constant weight. The weight of the residue is the total dissolved solids. Chemical characteristics were also measured {anions that were measured by Ion Chromatography (IC, DX-500 chromatography system) and cations and heavy metals were measured by ICP-OES instrument (Inductively Coupled Argon Plasma-Optical Emission Spectroscopy) (Perkin Elmer Optima-3000, USA)). Genotyping of founded bacteria was detected using Rapid Amplication of Polymorphic DNA (RAPD).

RESULTS

Microbiological examination of some patient's blood samples revealed that one patient had bacteraemia. The blood culture and bacteraemia was identified morphologically as *Pseudomonas* species (it made hemolysis to the blood in the blood agar medium and green florescent color in nutrient agar medium). A Gram stain showed that this bacteria was Gram negative bacilli. It was identified by biochemical reactions as *Pseudomonas aeruginosa*.

After 30 minutes of an HD session with a cuprophane membrane, IL-1β was significantly increased ($p < 0.01$ and $p < 0.001$) respectively in hemodialysis patients of unit I and II as shown in Figure (1).

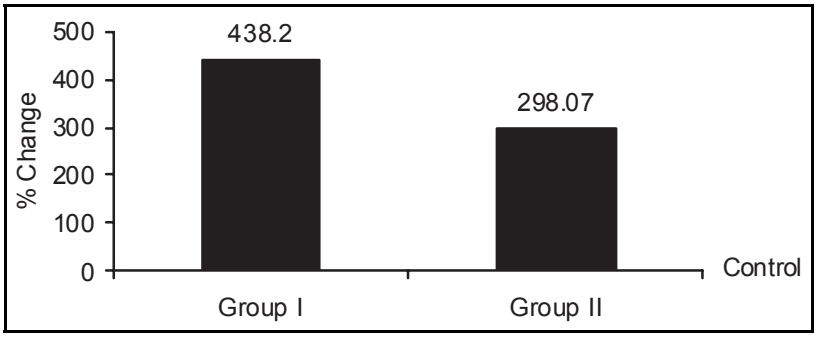

Fig. 1. Percent change of IL-1β levels in all patient groups.

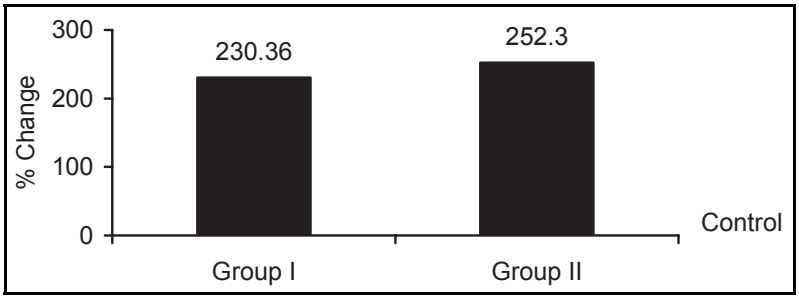

Fig. 2. Percent change of TNF-α levels in all patient groups.

The serum level of TNF-α was significantly increased ($p < 0.001$) in hemodialysis patients of unit I and hemodialysis patients of unit II ($p < 0.05$) compared to the healthy control group, as shown in Figure 2. The change in TNF-α level in Group I was statistically non-significant compared with that in Group II.

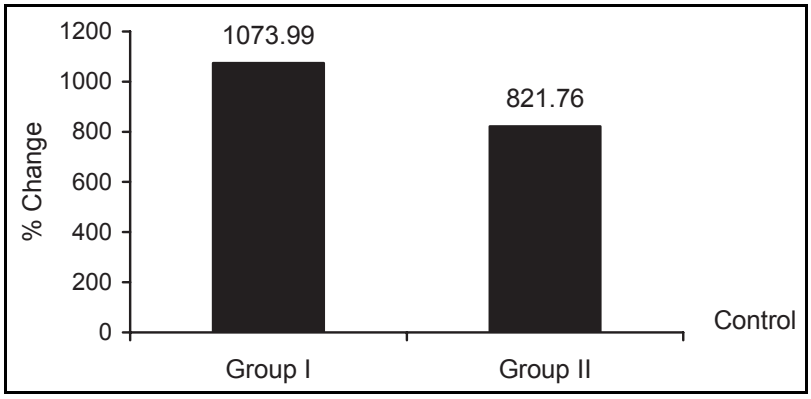

Fig. 3. Percent change of ferretin levels in all patient groups.

Serum ferretin was significantly increased ($p < 0.001$) in hemodialysis patients of both units (unit I and II) compared to the healthy control group as shown in Figure 3. Significant change ($p < 0.05$) was found between the two hemodialyzed patient groups (GI and GII).

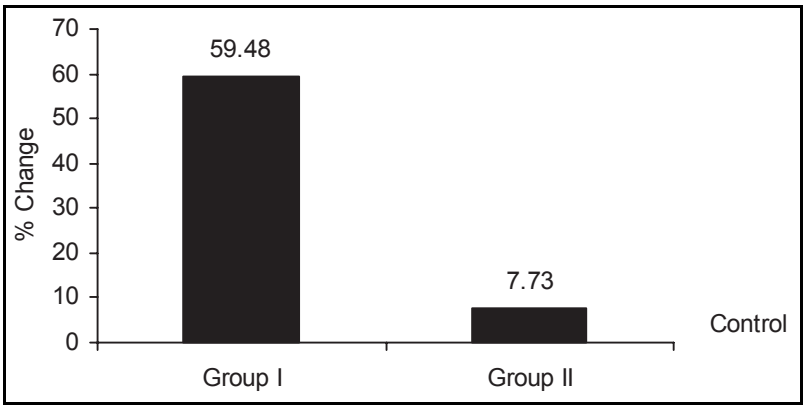

Fig. 4. Percent change of fibrinogen levels in patient groups.

The results shown in Figure 4 showed that plasma fibrinogen was significantly increased ($p < 0.001$) in hemodialysis patients of Group I compared to the healthy control group, whereas there was no significant change between hemodialyzed patients of GII and the healthy control group. Significance of $p < 0.05$ was observed between the two hemodialyzed patient groups (GI and GII).

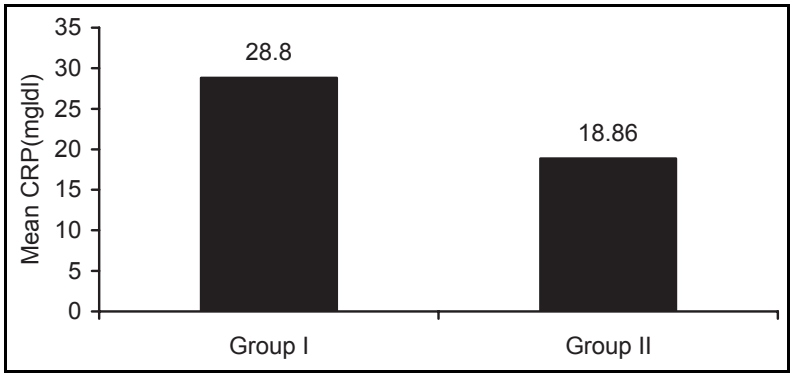

Fig. 5. Mean values of CRP levels in patient groups.

The results obtained in Figure 5 showed mean values of CRP in hemodialysis patients of both units (unit I and II) which are significantly increased ($p < 0.01$ and < 0.05) respectively compared to the healthy control group. The change in CRP level was non-significant between the two hemodialyzed patient groups (GI and GII).

Water samples results

Table 1. Physical characteristics of water samples.

Parameters	Unit(I) Tap water	Unit (II) Treated water	Unit (II) Tap water	Unit (II) Treated water	*ISO	Egypt
pH	7.4	6.8	7.4	6.9	--	6.8-7.4
**EC (μmohs/L)	295	22	256	20	--	< 300
***TDS (mg/L)	189	14	164	13	--	< 200

*ISO: International Organization for Standardization

**EC: Electrical conductivity.

*** TDS: Total dissolved solids.

Table 2. Chemical composition of water samples.

Parameters	Unit (I) Treated water	Unit (II) Treated water	ISO (mg/L)	Egypt (mg/L)
Ca	0	0	2	5
Mg	3	3	4	4
Na	15	8	70	70
K	1	1	8	5
Fe	< 0.01	0.07	--	0.1
Mn	0.02	0.02	--	0.1
F	0.12	0.1	0.20	0.2
Residual clorine	0.05	0.02	0.50	0.2
Cloramine	< 0.01	< 0.01	0.1	0.1
NH_3	< 0.2	< 0.2	0.	
SO_4	14	10	50	100
Cu	0.01	0.06	0.1	0.1
Ba	< 0.005	< 0.005	0.1	0.1
Zn	< 0.005	0.013	0.1	0.1
Pb	< 0.005	< 0.005	0.005	0.005
$CaCO_3$	4	2	--	10
Ag	< 0.005	< 0.005	0.005	0.005
Cr	0.010	0.008	0.014	0.014
Se	< 0.01	< 0.02	0.09	0.09
Al	0.004	0.002	0.01	0.01
Hg	< 0.001	< 0.001	0.002	0.002
Cd	< 0.001	< 0.001	0.001	0.001

Physical characteristics and chemical composition of water samples of the two hemodialysis units showed normal values of all parameters and were in agreement with ISO.

Table 3. Microbiological examination of water samples.

	Hetertrophic platecount (cfu/ml)	Total Coliform (cfu/100ml)	Fecal Coliform (cfu/100ml)	*Pseudomonas aeruginosa* (cfu/100ml)	Endotoxin (EU/mL)
Unit (I) Treated water	70	2	0	3	--
Unit (II) Treated water	40	1	0	0	--
Egypt	< 50	--	--	--	--
ISO 13959/2009	< 100	--	--	--	< 0.25

From each of the two dialysis units enrolled in the present study, samples of treated water were examined and showed total heterotrophic bacterial counts of 70 and 40 cfu/ml for unit (I) and unit (II), respectively (Table 3).

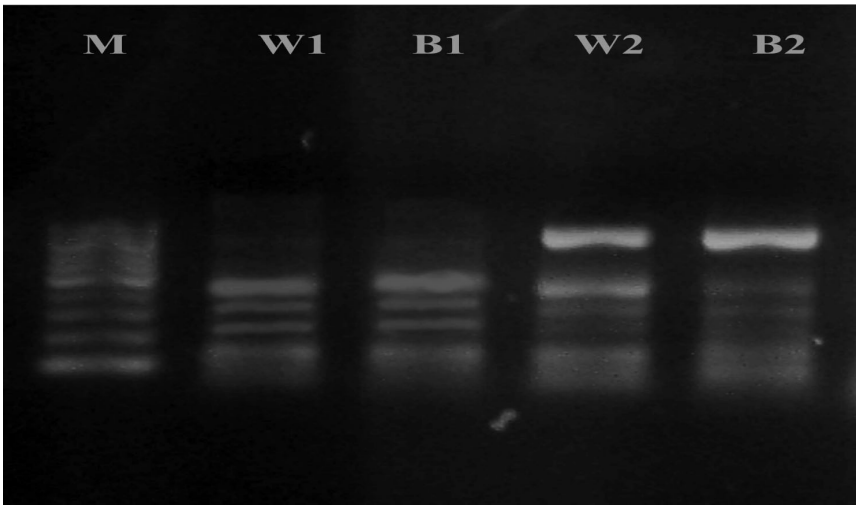

Photo 1. Electrophoretic patterns of water and blood *Pseudomonas aeruginosa* by RAPD PCR.

Photo 1 showed the electrophoretic pattern where column M contains the 100 bp DNA Ladder, and column W1 showed the RAPD amplicon of *Pseudmonus* isolate from water with primer NO 1. Column B1 showed the RAPD amplicon of *Pseudomonus* isolate from blood with primer NO 1. W2 showed the RAPD amplicon of *Pseudomonus* isolate from water with primer NO 2. Column B2 showed the RAPD amplicon of *Pseudomonus* isolate from blood with primer

NO 2. Electrophoretic data of RAPD PCR Assays revealed a similar pattern achieved by both primer 1 and 2, which means the two tested organisms had the same genotypic pattern on testing with RAPD PCR Assays.

STATISTICAL ANALYSIS

Statistical analysis was performed with the SPSSv 6.1 software package (SPSS Inc., Chicago, IL, USA). Each result was calculated as the mean ± standard error of the mean (SEM). Evaluation of the statistical significance of differences for paired and unpaired values was performed using the Student's t-test. Correlations were examined by linear regression analysis. Values of $P < 0.05$ were considered to represent statistical significance.

DISCUSSION

Chronic renal failure (CRF) is defined as an irreversible reduction in the glomerular filtration rate (GFR). Hemodialysis is the most common method used to treat advanced and permanent kidney failure (Vogt & Avner, 2004)

In hemodialysis, large amounts of water are used for diluting the concentrates to produce dialysis fluid. The water is produced on site by reverse osmosis units. Appropriate chemical and microbiological quality of the water is essential for dialysis patients. Dialysate purity has become a major concern in recent years since it has been proven that contamination of dialysate is able to induce the production of proinflammatory cytokines, putatively implicated in the development of dialysis related pathology (Nystrand, 2008).

To prevent dialysate-related microinflammation, it has been suggested to use dialysate with a microbiological quality of an intravenous pharmaceutical solution (Lonnemann, 2000; Panichi *et al.,* 1998).

Sometimes there is risk for serious catheter-related infection. This risk is avoided because of the regular use of a catheter-locking solution, with a dual action antithrombotic and antimicrobial venous port catheter device preventing the formation of biofilm and reducing the incidence of catheter-related bactremia. This finding corresponded to that founded and concluded by Allon (2003), and McIntyre *et al.* (2004).

Also, Ethylene oxide (ETO) has traditionally been the choice for sterilant for medical devices which are capable of inducing allergic reactions (Francos *et al.,* 1983). Acute hypersensitivity in sensitized patients can be limited by prolonged degassing and abundant rinsing of the extracorporeal circuit to get rid of residual ethylene oxide (Poothullil et al., 1975).

Our study was carried out to assess serum levels of some pro-inflammatory

cytokines and acute phase reactants which are associated with chronic inflammatory response in hemodialyzed patients and may be due to insufficient treatment for water used for hemodialysis. To this end, we measured IL-1β, TNF-α, Fibrinogen, Ferretin and C-reactive protein (CRP). Serum levels of IL-1β (14.8 ± 11.5pg/ml, 15.4 ± 11pg/ml, $p < 0.001$) and TNF-α (7.37 ± 6.9pg/ml, 5.45 ± 3pg/ml, $p < 0.001$) for unit I and unit II respectively were increased significantly in hemodialyzed patients compared with healthy control subjects IL-1β (4.48 ± 3.18pg/ml) and TNF-α (1.37 ± 1.16) (Fig. 1 and Fig. 2) These findings confirm findings in several studies (Malaponte *et al.,* 2007; Goldstein *et al.,* 2006; Rysz *et al.,* 2006 and Zoccali *et al.,* 2006). High levels of ferretin (1181 ± 500.17g/L, 927.3 ± 328.8g/L, $p < 0.001$) for unit I and II respectively compared with healthy control subjects (100.6 ± 56.5g/L) was also obtained (Fig. 3), which support previous conclusions that ferretin can be used as a marker of morbidity and mortality in hemodialysis patients (Kamyar *et al.*, 2001). Fibrinogen levels showed a highly significant increase (338.4 ± 137.2mg/dl) in patients of unit I compared with healthy controls.

This result is in agreement with conclusions that very high levels of fibrinogen in hemodialysis patients are secondary to the dual stimulation of inflammation and increased plasma volume (Kaysen *et al.,* 2003). The fibrinogen level of patients of unit II had no significance (279.3 ± 109.7mg/dl, $p < 0.001$) compared to the healthy control subjects (Fig. 4), as several parameters may affect albumin and/or fibrinogen metabolism in hemodialysis patients, such as nutritionalstatus, chronic subclinical or overt inflammatory state, metabolic acidosis, dialysis dose, and types and modalities of dialysis filter used (Giordano *et al.,* 2001). The extremely elevated CRP levels further demonstrate a chronic inflammatory state in a majority of patients (20.8 ± 23.6mg/dl, 18.8 ± 31.2mg/dl, $p < 0.001$) compared with healthy control subjects (Fig. 5) as hemodialysis patients have higher levels of serum CRP and fibrinogen levels which predict significantly the risk of mortality (Razeghi *et al.,* 2008).

In the present study, physical characteristics and chemical compositions of both water samples were in compliance with the water quality standards (Table 1 and Table 2). Total heterotrophic bacterial count was less than 100 cfu/ml, which is complys with ISO recommendations (Table 3). The presence of *Pseudomonas aeurginosa* and total coliforms were noticed in dialysis water samples which suggests the presence of biofilm on particulate sediment filters, which are used to remove sediment from water, as it was not changed for two months. This may have caused bacterial growth on the filter and led to subsequent bacteremia and/or pyrogenic reactions, or from the delivery loop. Also, hemodialysis units equipped with a storage reservoir for treated water (where the samples were taken) are more likely to experience contamination of

this water and distribution system because water treatment rooms and hemodialysis units are located far apart. The long piping involved makes it difficult to maintain minimal bacteria levels at all water outlets. One sample of patient blood indicated the presence of bacteria which was isolated and identified as *Pseudomonas aeurginosa*. Rapid Amplification of polymorphic DNA (RAPD) was used and proved that the two isolated *Pseudomonas aeurginosa* strains were the same and hac the same genotyping pattern (photo 1). This result suggests that bacteria may be transferred from water to patient blood through water treatment system or contaminated dialysate. Indeed, sporadic cases or small clusters of water-borne *Pseudomonas* septicaemia have been reported in the literature. *Pseudomonas aeruginosa*, P. maltophilia, and P. vesicularis were found in the blood cultures of patients with pyrogenic reactions, and the micro-organisms were also isolated from tap water and the effluent of reprocessed dialysers (Vanholder *et al.,* 1990), and may explain the importance of water as a source of bacterial infection and one of the reasons of inflammation in hemodialysis patient. This finding is in agreement with others that have found that these bacteria release pyrogenic substances such as endotoxins, peptidoglycans, exotoxins and fragments thereof. Pyrogens derived from contaminated dialysate either alone or in costimulation with activated complement components are the most important activators of circulating mononuclear cells in patients on chronic intermittent hemodialysis. Activated mononuclear cells release pro-inflammatory cytokines which are key mediators in acute and chronic inflammatory diseases associated with long-term hemodialysis therapy (Lonnemann, 2000). Also, upgrading water systems from specified ISO on clinical measures associated with inflammation in dialysis units are reflected in improvement in markers of inflammation (Rahmati *et al.,* 2004).

In conclusion, dialysis water which met the standards set by legislation was actually found to be contaminated by pathogenic bacteria. Also, serum inflammatory cytokine levels (IL-1β and TNF-α), ferretin, fibrinogen and C-reactive protein (CRP) shower higher serum values than normal subjects.

RECOMMENDATIONS

1 - Standards must include new methods for measuring bacteriological contaminants in addition to colony-forming units, and all contaminants received by patients, whether biological, chemical, or physical, must be considered.

2 - In view of these results, it is suggested that endotoxin testing become a part of regular water quality control in dialysis.

3 - The use of sterile dialysate and endotoxin-free water may reduce cytokine

production and plasma levels of acute phase proteins, and may positively influence progressive inflammatory disease with end stage renal failure.

4 - Particulate filters which are used to remove sediment from water should be regularly replaced and disinfected according to the manufacturer's recommendations

5 - Microbiological surveillance should be done monthly, on a regular basis.

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درجة نقاء المياه وحدوث الالتهابات في مرضى الاسترشاح الكلوي

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خلاصة

يعاني مرضى الفشل الكلوي المزمن الذين يعالجون بالاسترشاح الكلوي بصفة منتظمة من خطر العدوى الميكروبية بالدم والتي تعرضهم للمخاطر سواء كان سبب العدوى داخلي (يوجد في المريض) أوخارجي (ماء الاسترشاح الكلوي – أجهزة الاسترشاح الكلوي). كذلك يؤثر غشاء الاسترشاح الكلوي في عملية مرور البكتريا إلى دم المريض من خلاله والتي بمجرد وصولها دم المريض تحفز إفراز المنشطات الخلوية التي تصاحب الالتهاب الناتج عن مرورها خلال ماء الاسترشاح.

استهدفت الدراسة قياس مستوى بعض السيتوكينات المرتبطة بحدوث الالتهابات مثل: (إنترليوكين – 1 بيتا $IL-1\beta$، عامل النخر المسبب للسرطان – ألفا $TNF-\alpha$) بالإضافة إلى بعض البروتينات المتكونة خلال تفاعلات الطور الحاد acute phase reaction مثل (بروتين سي النشط CRP والفريتين والفيبرينوجين) التي تزيد مع حدوث الالتهابات. أثناء عملية الاسترشاح الكلوي وقد وجد ارتفاعاً معنوياً في نسبة بروتين سي النشط في مرضى الاسترشاح الكلوي مقارنة بالأصحاء. كما زادت تركيزات الإنترليوكين – 1 بيتا، عامل النخر المسبب للسرطان – ألفا، الفريتين وكذلك الفيبرينوجين. بينما قلت معدلات الألبيومين والهيموجلوبين وكذلك أوضحت الدراسة حدوث تفاعلات ناتجة عن خلايا monocyte والتي قد تحدث نتيجة لتلوث مياه الاسترشاح الكلوي بالبكتريا.

بالنسبة لماء الاسترشاح الكلوي جاءت نتائج الخواص الطبيعية والتحاليل الكيميائية مطابقة للمعايير الدولية وكذلك قوانين وزارة الصحة المصرية. بينما أسفرت التحاليل

الميكروبيولوجية عن وجود بكتريا (Pseudomonas aeruginosa) مما يشكل خطراً على صحة المرضى الذين ظهرت عليهم أعراض الإصابة البكتيرية، وأظهرت المزرعة إصابة المريض ببكتريا Pseudomonas aeruginosa والتي أوضحت الدراسة وجودها في ماء الاسترشاح الكلوي أيضاً. وأثبت اختبار (RAPD) الخاص بجينات الحامض النووي البكتيري أن العزلتين البكتيريتين من ماء الاسترشاح ودم المريض لهم نفس الوزن الجزيئي والمحتوى الجيني مما يحتمل انتقال البكتيريا من الماء إلى المريض. كذلك أسفرت الدراسة عن إمكانية استخدام بعض السيتوكينات (الإنترليوكين -1 بيتا، عامل النخر المسبب للسرطان-ألفا) بجانب اختبار الفريتين، الفيبرينوجين وبروتين سي النشط كأحد العوامل التشخيصية التي يمكن التنبؤ عن طريقها بحدوث الالتهابات للمريض. خلصت الدراسة إلى ضرورة وضع معايير شديدة لضبط جودة مياه الاسترشاح الكلوي والتي يمكن أن تكون مطابقة للمعايير الحالية وهي الوحدة المكونة للخلية بينما تحتوي على البكتريا الممرضة كذلك أوصت الدراسة بعمل دراسات مقارنة لاستكشاف دور ضعف نقاء المياه في حدوث الالتهابات وكذلك ارتفاع المؤشرات الخاصة بها.