Role of Online Hemodiafiltration in Improvement of Inflammatory Status in Pediatric Patients With End-stage Renal Disease

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Introduction. Patients with end-stage renal disease are known to suffer from chronic inflammation as the result of an ongoing subacute cytokine induction, which may contribute considerably to dialysis-related long-term morbidity and mortality. In order to assess the inflammatory risk associated with online hemodiafiltration compared to conventional hemodialysis, we compared the cytokine induction profile of pediatric patients during treatment with each these modalities of dialysis.

Materials and Methods. Thirty pediatric patients on regular hemodialysis for at least 6 months were shifted to online hemodiafiltration. We collected serum samples before and 6 months after initiation of online hemodiafilration. The target pro-inflammatory cytokines selected were interleukin-6, tumor necrosis factor-α, and high-sensitivity C-reactive protein.

Results. High-sensitivity C-reactive protein decreased significantly on hemodiafiltration. The mean C-reactive protein level after 6 months was 3.41 µg/mL in the online hemodiafilration as compared to 7.98 µg/mL in the hemodialysis group (P = .01). Plasma interleukin-6 and tumor necrosis factor-α and tumor necrosis factor-α also decreased significantly on hemodiafiltration and the values were 100.44 pg/mL versus 168.40 pg/mL (P = .002) and 11.45 pg/mL versus 15.70 pg/mL (P = .008), respectively, for the hemodiafilration and hemodialysis groups.

Conclusions. Online hemodiafiltration is associated with dampened pro-inflammatory cytokine profile compared to conventional hemodialysis in children with end-stage renal disease.

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INTRODUCTION

The incidence of the chronic kidney disease (CKD) in children is increasing steadily. Children with end-stage renal disease (ESRD) have a 10-year survival rate of around 80% and an age-specific mortality rate 30 times higher than in children without ESRD.[1] The leading causes of death in children with CKD are cardiovascular disease (CVD) and infection.[1,2] These patients also demonstrate clear clinical features of ineffective immune response, which is depicted by the low rate of successful immunization, poor response in tuberculin tests, low rejection rate, and high frequency of infections which promotes a state of chronic inflammation.[3]

Inflammation is a strong predictor of mortality in dialysis patients.[4] The evidence of sustained low-grade inflammation is commonly observed and enhanced in CKD stage 5, and following the initiation of dialysis therapy, despite the clinical stability of the patient.[3] In general, the prevalence of low-grade inflammation in the pediatric CKD population appears to be much higher than in the general pediatric population.[5]

Patients on dialysis frequently demonstrate dialysis-related complications, such as dialysis solution or membrane bioincompatibility, uremic toxicity, and chronic infections that may contribute to a state of chronic inflammation.[3] The most commonly used marker of inflammation in the clinical practice is an elevated serum concentration of C-reactive protein (CRP), an acute phase reactant, which is also a sensitive and independent marker of anemia, malnutrition, and cardiovascular mortality. C-reactive protein is an inflammatory mediator that modulates endothelial cells, macrophages, and vascular smooth muscle cells in the process of atherogenesis. The higher the CRP level is, the greater the CVD risk will be in ESRD patients.[3] Serum levels of CRP appear to reflect the generation of pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α), which are all markedly elevated in ESRD patients and predict mortality.[3] Following tissue damage, both the innate and the adaptive immune systems are triggered in the face of invasion by an infectious agent or antigen challenge. This complex response is fundamentally what is named inflammation, which when uncontrolled lead to further tissue damage. The development of inflammatory reactions is controlled by products of the plasma enzyme systems (such as complements, coagulation factors, kinin, and fibrinolytic pathways), cytokines (such as IL-1, IL-6, TNF-α, and transforming growth factor-β), vasoactive mediators (histamine and serotonin), and prostaglandins and leukotrienes.[4] Cytokines interact with high-affinity cell membrane receptors and regulate the transcription of certain cellular genes, resulting in cell behavioral changes. Consequently, cytokines transform their information signal on target cells in the different tissues (ie, vascular tissue, muscle, brain, and liver), leading to well-known complications of CKD. Thus, a model of chronic stimuli to the inflammatory response is created, in which there is interaction between highly prevalent chronic infections, fluid overload and accumulated uraemic toxins, dialysis membrane and dialysis fluid bioincompatibility.[6]

Hemodiafiltration is the optimum blood purification therapy to provide significant removal of uremic solutes beyond range of small molecules, through a highly permeable dialysis membrane that combines diffusive and convective solute transport.[7] Convective transport requires ultrafiltration of fluid exceeding the desired weight loss. The replacement fluid should be sterile and pyrogen-free with similar composition to plasma water. Online preparation of a sterile nonpyrogenic ultrapure dialysis fluid ensures a safe and highly effective therapy.[7] Hemodiafiltration using ultrapure replacement fluid provides both effective cytokine removal through high-volume convection and dampened inflammatory stimulation through better microbiological control, which results in lower levels of serum inflammatory cytokines, with reduced serum CRP and IL-6 level.[8,9] In order to assess the inflammatory risk associated with online hemodiafiltration in contrast to conventional hemodialysis, we compared the cytokine induction profile of ESRD pediatric patients during treatment with each modality of dialysis.

MATERIALS AND METHODS

This is a single-center cohort study performed in the hemodialysis unit of the Centre of Pediatric Nephrology and Transplantation, Children's Hospital, Cairo University. The study included 30 patients who had been treated by conventional hemodialysis for at least 6 months, who were switched to online hemodiafiltration with a follow-up period of another 6 months thereafter. Patients with acute or chronic infectious diseases and those nonadherent to treatment or medical advice were excluded from the study.

All patients received 3-time weekly dialysis treatments. Online hemodiafiltration was done using the Fresenius 4008 dialysis system (Fresenius Medical Care, Hesse, Germany) with the same hemodialysis configurations, same surface areas of the polysulfone dialysis membranes during online hemodiafiltration, identical blood flow rates, dialysis solution flow rates (500 mL/min), and dialysis solution temperature of 36°C used during both conventional hemodialysis and

online hemodiafiltration. During both treatment modalities, bicarbonate was provided from powder cartridges using the biBAG system (Fresenius Medical Care, Hesse, Germany).

Ultrapure water was used for the preparation of bicarbonate-containing dialysis fluid, which undergoes 1 step of ultrafiltration, converting it into ultrapure dialysis fluid. The substitution fluid was prepared continuously from the dialysis fluid by one additional step of controlled ultrafiltration, before it was infused prefilter (predilution) into the blood, with re-infusion rate of two-thirds of or equal to blood flow rate guided by the transmembrane pressure to be kept below 200.

The online system, ONLINE plus (Fresenius Medical Care, Hesse, Germany) is integrated into the dialysis machine (4008 series) and consists of two ultrafilters (DIASAFE plus), an infusate pump module, and disposable infusate lines. Both filters are subjected to automated membrane integrity tests before dialysis, and are replaced after 100 treatments or 12 weeks of use, whichever comes first.

Clinical data were collected and venous blood samples were drawn before the initiation and at the end of the study period. Samples were centrifuged at 3500 rpm for 10 minutes, the serum was separated and immediately stored at -20°C until analysis. In addition to routine biochemical parameters, serum IL-6, TNF-α, and high-sensitivity CRP were assayed using commercially available immunoenzymatic methods in the Laboratories of Clinical and Chemical Pathology department, National Research Centre.

Blood pressure index was calculated by dividing patient's systolic and diastolic blood pressure measurements by the 90th percentile of systolic and diastolic blood pressure, respectively, using blood pressure charts appropriate for the patient's age and sex. Patients were considered hypertensive if their blood pressure index was more than 1.

Data were expressed as means ± standard deviation for normally distributed data, range and median for non-normally distributed data. Data were analyzed by using paired *t* test for normally distributed continuous variables and the chi-square tests test for categorical variables. The Pearson correlation test was used for evaluating correlation between variables. The SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, Ill, USA) was used to obtain the descriptive and analytical statistics. *P* values less than .05 were considered significant.

RESULTS

The mean age of the patients was 11.3 ± 3.2 years (range, 4 to 17 years); 12 boys (40%) and 18 girls (60%). The mean duration on hemodialysis was 53 ± 32 months (range, 6 months to 12 years). Chronic kidney failure were attributed to uropathies (urethral stricture, ureteropelvic junction obstruction, vesicoureteral reflux, and posturethral valve) in 9 cases (30%), nephronophthisis in 6 cases (20%), focal segmental glomerulosclerosis in 4 cases (13.3%), hereditary nephropathies in 3 cases (10%), chronic hemolytic uremic syndome and chronic interstitial nephritis in 1 case each, and undetermined in 6 cases (20%).

There was no significant changes in the mean predialysis mean blood pressure index (1.00 ± 0.13 on hemodialysis versus 1.00 ± 0.11 on hemodiafiltration; *P* = .75). Also, there was no

Biochemical and Hematological Data of Patients Who Switched to Online Hemodiafiltration

Parameter	Hemodialysis	Online Hemodiafiltration	*P*
Hemoglobin, g/dL	10.9 ± 1.4	11.0 ± 1.3	.60
Hematocrit, %	32.5 ± 4.0	33.3 ± 3.5	.45
Serum calcium, mg/dL	9.00 ± 0.89	8.97 ± 1.00	.90
Serum phosphorus, mg/dL	5.31 ± 1.67	4.85 ± 1.62	.27
Calcium-phosphorus product, mg/dL	48.0 ± 16.5	44.2 ± 17.8	.38
Alkaline phosphatase, IU/L	778.0 ± 634.0	821.5 ± 842.8	.82
Blood urea nitrogen, mg/dL	75.0 ± 24.0	62.5 ± 16.9	.02
Serum creatinine, mg/dL	6.80 ± 1.76	6.70 ± 1.36	.79
KT/V	1.78 ± 0.48	1.82 ± 0.35	.76
Serum albumin, g/dL	3.55 ± 0.30	3.66 ± 0.41	.24
Serum sodium, mmol/L	135.10 ± 2.51	135.40 ± 1.92	.73
Serum potassium, mmol/L	5.85 ± 0.95	5.71 ± 0.87	.53

significant changes in the mean postdialysis mean blood pressure index (0.95 ± 0.10 on hemodialysis compared to 0.96 ± 0.13 on hemodiafiltration; $P = .64$). Comparison of the mean blood pressure index during dialysis session showed that there was no significant difference during hemodialysis versus during online hemodiafiltration (-10.7 ± 9.2% versus -10.37 ± 10%; $P = .89$).

A significant decrement in plasma high-sensitivity CRP was detected after 6 months of online hemodiafiltration (7.98 ± 9.00 µg/mL versus 3.40 ± 2.88 µg/mL; ($P = .01$), as well as a decrement in plasma IL-6 (15.7 ± 5.9 pg/mL versus 11.5 ± 6.0 pg/mL; $P = .008$) and plasma TNF-α (168.4 ± 84.9 pg/mL versus 100.4 ± 75.6 pg/mL; $P = .002$).

DISCUSSION

Many active inflammatory processes influence the outcome of ESRD patients and may result in atherosclerosis and CVD.[3] The incidence of CVD should be lowered and survival rates improved when it is possible to reduce the vascular inflammation, which is evidently reduced by hemodiafiltration. We chose 3 pro-inflammatory cytokines (CRP, IL-6, and TNF-α), all of which are strongly associated with vascular inflammation, to study the effect of online hemodiafiltration on the patients' inflammatory status. Being a risk marker for CVD both in the general population and in ESRD patients, CRP was found to be significantly reduced after 6 month of hemodiafiltration in our study. Similarly, IL-6, which plays a key role in regulating humoral and cellular responses and is produced by many vascular cells, which are highly associated with vascular inflammation, was significantly reduced after 6 month of hemodiafiltration. Ghani and colleagues revealed a direct link between IL-6 levels and cardiovascular morbidity and mortality.[10] Kuo and colleagues studied the effect of 6 months of hemodiafiltration on pro-inflammatory cytokines in adults.[11] Their results showed a decrement of the plasma levels of IL-18 and TNF-α, but not IL-6 and CRP.[11] A similar study by Pedrini and coworkers on 69 Italian adult patients showed lowered levels of CRP on hemodiafiltration (5.5 ± 5.5 mg/L versus 6.7 ± 6.1 mg/L, $P = .03$).[12] Tumor necrosis factor-α has a major role in CVD pathogenesis and has been detected in human atherosclerotic plaques. Increased levels of TNF-α have been observed after acute myocardial infarction. Tumor necrosis factor-α regulates the synthesis of fibrinogen and factor VIII, which are risk factors for atherosclerosis. Kuo and coworkers showed that it can be removed in high volume continuous renal replacement therapy.[11] We believe that TNF-α can also be removed by convection in hemodiafiltration. In our patients, there was a significant reduction of plasma TNF-α 6 months after online hemodiafiltration.

The impact of hemodiafiltration on serum inflammatory cytokines levels may result from cytokine removal, due to high volume convection, or reduced inflammatory stimulation secondary to better microbiological control in hemodiafiltration. Similar results were provided by Schiffl and colleagues in 2001, where ultrapure dialysis lowered down the inflammatory stimulation with reduced serum CRP and IL-6 levels after 12 months,[9] and also by the Dialysis Outcomes and Practice Patterns Study in 2006, which has shown that high-efficiency hemodiafiltration reduces mortality risk which may be an effect of cytokine reduction.[13] A study by Sawires and colleagues, which examined the effect of high-flux membranes on adhesion molecules, showed that by lowering levels of TNF-α, the levels of the adhesion molecules intercellular adhesion molecule 1 and vascular cell adhesion protein 1, which are an essential part in the process of leukocyte migration, were lowered.[14]

Hemodiafiltration is a highly effective dialysis modality expanding the spectrum of removed uremic toxins from small to middle-sized molecular solutes. In addition, the online hemodiafiltration using high fluid substitution allows a greater clearance of large uremic toxins; the micro-inflammation status observed in CKD patients is associated with endothelial damage; and amelioration of the chronic micro-inflammation using high convective transport appears to reduce endothelial damage and promote endothelial repair.[12]

Online hemodiafiltration, being able to provide the largest removal of the widest range of solutes among available dialysis therapies, has a better ability to remove urea than hemodialysis as shown by improvement of blood urea nitrogen levels, which showed significant reduction during online hemodiafiltration as compared to hemodialysis. Although our study rendered informative results, limitations in the sample size and longitudinal effect exist, because of reasons related to financial

limitations and capacity of our dialysis unit after applying the aforementioned exclusion criteria.

CONCLUSIONS

Using online hemodiafiltration technique that combines the use of high-flux synthetic membrane with low bioreactive profile and the use of ultrapure dialysis fluid, showed a significant reduction of pro-inflammatory cytokines in children with ESRD. The long-term impact of the technique requires to be studied through long-term follow-up of these patients treated with online hemodiafiltration to confirm its role in improving the inflammation, malnutrition, and cardiac disease as predictors of mortality in hemodialysis patients.

CONFLCIT OF INTEREST

None declared.

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