TYPE OF RENAL REPLACEMENT THERAPY (HEMODIALYSIS VS PERITONAL DIALYSIS) DOES NOT AFFECT CYTOKINE GENE EXPRESSION AND CLINICAL PARAMETERS OF RENAL TRANSPLANT CANDIDATES

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OBJECTIVE

Patients with end-stage renal disease suffer from immune disturbances, caused by uremic toxins and influenced by dialysis treatment. Uremia was reported to influence cytokine synthesis. Abnormal function of regulatory T-cells in uremia as well as an imbalance of immature and memory B cells have been reported. The above described abnormalities are further enhanced by renal replacement therapy. It is not clear whether type of renal replacement therapy (RRT) affects gene profiles in patients on active transplant waiting list.

The aim of the study was to investigate pre-transplant blood cytokine and apoptosis related gene expression in patients on hemodialysis (HD) and peritoneal dialysis (PD). We focused on the immune factors known to be related to tissue injury and inflammation (IL-6, IL-8, IL-18, NGAL and TNF-alpha), apoptosis (FAS, CASP3, TP53) and Th1 lymphocyte activation (IFN-gamma, IL-2), as well as Th2 (IL-10, TGF-beta), and regulatory T cells function (FoxP3).

METHODS

87 renal transplant candidates (aged 16-72y., mean 47y., 34F/53M) were included in the study. Among them 66 patients were treated with hemodialysis and 21 patients with peritoneal dialysis. Ambulatory HD patients were hemodialyzed on polysulfone membrane dialyzers with bicarbonate-containing solutions for 4 h three times weekly. Peritoneal dialysis solutions of 2 l with various concentrations of glucose were used four times daily. They were on renal replacement therapy from 1 to 97 months (mean 25± 18 months).

Blood samples for routine laboratory tests as well as gene expression were taken during immediate pre-transplant examination before introducing of immunosuppressive therapy. The peripheral blood samples were obtained with PAXgene blood RNA tubes, RNA was isolated with PAXgene Blood RNA kit (PreAnalytics) and reversely transcribed with high capacity RNA to cDNA kit (Applied Biosystems). The peripheral blood gene expression of caspase-3, Fas, p53, FoxP3, IFN-gamma, IL-2, IL-6, IL-8, IL-10, IL-18, NGAL, TGF-beta, and TNF-alpha were assessed with the real-time PCR on custom-designed low density arrays (Taqman). The expression data are presented as $\Delta Ct = Ct_{gene} - Ct_{GAPDH}$, where Ct is the cycle threshold value and defines the calculated cycle number, in which the fluorescence measured during PCR reaction increases over the preset threshold value. The relative change in the observed expression between the groups is calculated as $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \text{mean}\,\Delta Ct_{HD} - \text{mean}\,\Delta Ct_{PD}$.

RESULTS

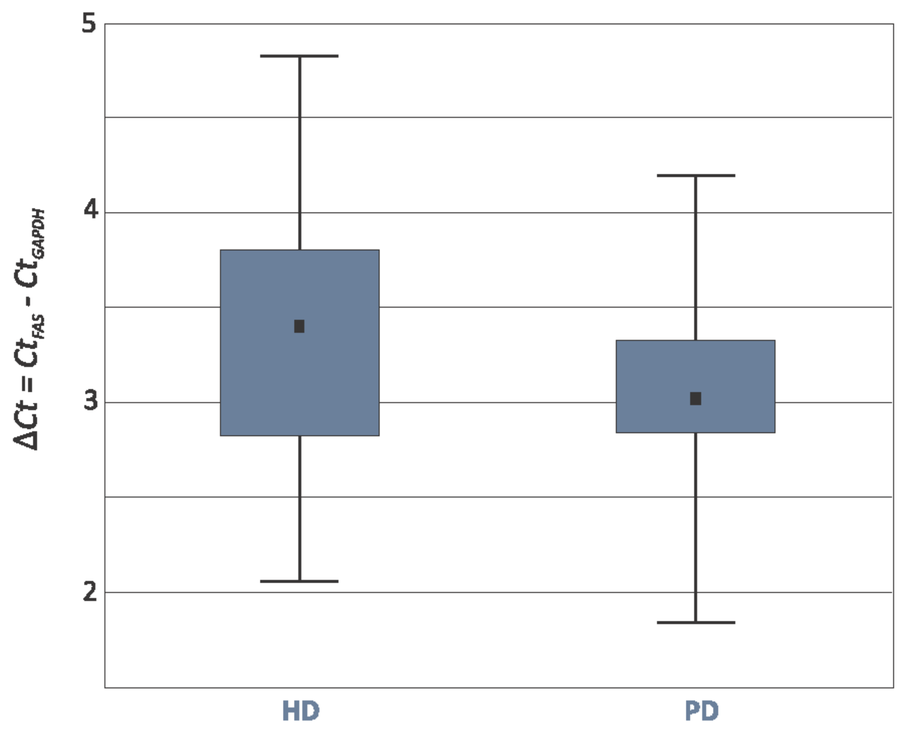
The mean serum expression of examined genes showed no significant differences between the PD and HD with the exception of FAS, which expression was increased 1.3x in PD patients compared to HD group ($p<0.05$). (Figure 1)

There was a trend to increased level of pro-inflammatory cytokines expression in PD group. The clinical inflammatory parameters (CRP, albumin, cholesterol, haemoglobin levels) did not differ between the groups. Only pre-transplant serum uric acid concentration was significantly higher in PD group (6.8 vs 5.4 mg/dL, $p<0.05$) (Table 1)

Table 1. Subcject characteristics

	HD	PD	
Recipient age (years, mean ± SD)	48 ± 12	42 ± 14	ns
Recipient gender (female/male)	39/27	14/7	ns
Time of dialysis (months, mean ± SD)	33 ± 42	15 ± 10	p<0.05
BMI (kg/m^2)	24.6 ± 3.6	24.8 ± 3.7	ns
Primary kidney disease:			
Diabetic nephropathy	4	5	
Chronic glomerulonephritis	22	12	
Hypertensive nephropathy	7	1	
Polycystic renal disease	9	1	
Chronic interstitial nephritis	16	0	
Other/unknown	9	4	
Clinical pretransplant parameters			
Hgb	11.1 ± 1.7	10.8 ± 1.4	ns
WBC count	7.4 ± 2.3	7.5 ± 3.3	ns
PLT count	198 ± 64	230 ± 65	ns
CRP	6.9 ± 7.0	3.2 ± 4.6	ns
Cholesterol	181 ± 57	206 ± 40	ns
Albumin	4.2 ± 0.8	3.9 ± 0.8	ns
Creatinine	7.0 ± 2.0	7.4 ± 2.8	ns
Uric acid	5.4 ± 2.8	6.8 ± 1.4	p<0.05

Figure 1. Observed ΔCt values for FAS expression versus GAPDH in the two studied groups.



CONCLUSION

Type of the renal replacement therapy exert no effect on cytokine gene expression as well as inflammatory clinical parameters. Possibly uremia is the most potent factor, which triggers immune activation and further changes induced by type of renal replacement therapy are too subtle to be apparent.

FUNDING SOURCE

This study was supported by research grant from the Polish Society of Nephrology.